ACTIONS OF THYROID HORMONE ON MYOCARDIAL CONTRACTILITY IN THE INTACT ANIMAL

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University of Cape Town

To my wife Elizabeth,
my Parents,
and my Teachers.

P R E F A C E.

To the uninitiated the conduct of Research implies a process whereby answers to questions are obtained by definitive experimentation and analysis; after the completion of this work it would seem more appropriate to define Research as the process whereby questions are generated. Whereas it is true that questions were set and experiments designed to prove or disprove these, in the final analysis it appeared that for every answer provided several new questions were found to have arisen and new experiments were required to answer some of these; others have remained as questions.

The experiments described herein were performed during the tenure of the Hermann Blumgart Research Fellowship at Harvard Medical School and the Beth Israel Hospital, Boston, Massachusetts. I wish to thank the Women's Auxiliary of the Massachusetts Chapter of the American Heart Association for providing the support for this Fellowship.

These experiments were all performed and the data analysed by myself. Miss Joan M. Burke, B.S. provided technical help and performed the serum cholesterol determinations. Without her enthusiasm and unfailing help this work would never have been completed in the two years available.

The programme-directors were Drs. A. Stone Freedberg and George S. Kurland of the Department of Medicine, Beth Israel Hospital, Boston. For their persistent support, advice and encouragement I shall always be grateful. In retrospect I wish to thank them both particularly for refusing to accept any of my ideas until they were conclusively proven by the experiments.

This thesis has a considerable proportion of it devoted to the description of equipment, methods and statistical analysis. Since the methodology was of crucial importance, it was felt that it was justified to devote a section of the body of the text to this rather than insert it in the Appendix. The statistical method chosen was an analysis of variance; the versatility of the procedure is illustrated also by a detailed treatment in the body of the text. In this regard my gratitude is expressed to Dr. Theodore S. Colten, Sc.D., Assistant Professor of Statistics at Harvard School of Public Health for his unfailing patience in providing what amounted to an individually instructed course in Biostatistics.

Throughout the test liberal use is made of graphic illustrations of the haemodynamic principles discussed. In order to achieve this numerous figures from the work of other authors had to be used, with apologies. The sources of all "borrowed" figures are indicated. A word of thanks to Mr. Paul Showstark of the Department of Biophotography of the Beth Israel Hospital for performing the photography of the graphs arising out of these experiments.

A final word of thanks to Miss L. Malan, who as typist, tackled and executed successfully a formidable task.

W.F.L.

I N D E X.

"Die Methode ist Alles"

Carl Ludwig.

The central role of the mammalian heart in the circulation was recognised by Nei Chung when he wrote in his Third Book of the Yellow Emperor's Classic of Internal Medicine (2698 - 2598 B.C.):

Treatise on the Five Viscera in Relation to their part in Perfecting Life,

"The Heart is in accord with the pulse. The complexion of a person shows when the heart is in a splendid condition. The heart rules over the kidneys ....."(1)

The importance of assessing the functional state of the heart was realised even by the early Egyptian physicians in the 17th Century B.C. as described in the Ebers Papyrus:-

"The beginning of the physicians secret : knowledge of the heart's movements and knowledge of the heart. There are vessels from it to every limb. As to this, when any physician, any surgeon, or any exorcist applies the hands or his fingers to the head, to the back of the head, to the hands to the place of the stomach, to the arms or to the feet, then he examines the heart, because all his limbs possess its vessels, that is, the heart speaks out of the vessels of every limb".(2)

The role of left ventricular systole in the generation of force necessary to propel blood through the vascular tree, was appreciated by Stephen Hales in his observations on the roles

of systole and diastole in the determination of peripheral flow.(3)

The diversity of factors which may act as determinants of left ventricular function was alluded to by Jean Poiseuille in his "Researches upon the Force of the Left Ventricle":

> "The course of the blood is so subject to the action of the heart, that new researches upon the force of this organ have seemed to us worthy of the entire interest of physiologists.
>
> One should say that the age, the sex, the temperament, the idiosyncrasies, the state of consciousness, of sleep, of exercise, of rest, of health, of illnesses, the passions, modify more or less the force of the heart. We recognise the influence of these modifying agents; but does their existence prescribe all effort which would have for its aim the determination of the limits of this force?"(4)

A great deal of interest has since focussed on the factors that may govern not only the basal level of function of the heart but also its ability to increase its energy output according to the requirements of stress. The mechanisms by which the heart can accomplish an increase in force and output have been defined. Not only can the heart function at an increased rate of contractions, but it can increase its output during each stroke by increasing the force of contraction. Both intrinsic and extrinsic factors have been shown to influence the state of performance, or contractility, of the

myocardium.

Although a definition of myocardial "contractility" in terms of haemodynamic principles will be attempted later, its meaning will be indicated at this stage by considering, in perspective, its role in the function of the heart as an entire organ. A fundamental characteristic of the heart is its ability to readily alter its state of performance. This ability, whether induced by increased frequency of contraction, increased sympathetic background or altered filling, provides for modulation of the work and power of the heart necessary to function at a speed commensurate with the time available. Whatever the manner in which it is ultimately brought about, it is an alteration in myocardial "contractility" that underlies the flexibility of the functional state of the heart.

Little is known about the contractile state of the myocardium in clinical thyroid disease; whether the altered myocardial physiology is due to a primary action of the hormones or secondary to the peripheral vascular and metabolic actions; to assess this directly, left ventricular catheterisation is necessary but this is not justifiable in patients with thyroid disease. Thus far indirect assessment of contractility has had to be made from information obtained by means of arterial and venous cannulations. The purpose of this thesis is to review the factors known to influence myocardial contractility, to outline the estimation of this aspect of myocardial function in the intact animal and to present evidence obtained by direct measurement of myocardial function in dogs with experimentally-induced hypo- and hyperthyroid states that myocardial contrac-

tility varies according to the thyroid state of the animal. Interactions between catecholamines and thyroid hormone on myocardial contractility are assessed and the direct effect of the hormone on the myocardium defined. Mechanisms of action of the hormone on the function of the heart are suggested and the implications of the observations obtained in these experiments in the genesis of thyrocardiac disease states are discussed.

2. FACTORS THAT DETERMINE THE LEVEL OF MYOCARDIAL PERFORMANCE.

Exercise has customarily been used as a model stimulus for both the rate of action and the level of performance of the heart. Most research into factors involved in the control of cardiac function in the intact animal has concerned itself with a detailed analysis of the mechanisms responsible for the physiological alterations induced by exercise.

A. EXTRINSIC TO THE MYOCARDIUM:

(a) HUMORAL:

(i) The Catecholamines: Extrinsic humoral influences have been confined to possible roles played by the circulating catecholamines, epinephrine and norepinephrine. Increased secretion of catecholamines by the adrenal gland during exercise has been generally assigned an important role in mediating the cardiac response to stress and exercise. However, intravenous infusions of both physiological and pharmacological amounts of both l-epinephrine and norepinephrine, have failed to reproduce the left ventricular response to exercise both in terms of rate and force of contraction.(5) The onset of the response to these hormones was quite gradual, leading to a bradycardia rather than tachycardia as infusions were continued. In these same animals, conscious and unrestrained with indwelling monitoring equipment, exercise produced a response quite unlike that obtained with either epinephrine or norepinephrine. It is only in the isolated heart preparation(6) and in the anaesthetized(5,7) or vagal-blocked animal(8,9) that these hormones cause an increase in heart rate with the increase in contractile force.

Urinary excretion of epinephrine and norepinephrine is

nevertheless found to be increased during muscular work, even with the mere assumption of the erect posture.[10]

As a result of their extensive experimentation in conscious, unsedated dogs, Rushmer et al[11] came to the conclusion that the left ventricular responses induced by physiological levels of l-epinephrine and norepinephrine are relatively unimportant in effecting the cardiovascular responses to exercise.

(ii) Glucagon: The demonstration by Farah and Tuttle[12] that glucagon had inotropic and chronotropic actions similar to those of epinephrine on isolated heart preparations, has raised the question of whether this hormone has a physiological myocardial action. The subsequent findings of Lucchesi[13] and Glick et al[14] in the intact animal have established that the hormone acts very similarly to the catecholamines by stimulation of the beta-receptor, but that its action can occur in the presence of beta-adrenergic blockade. The suggestion has been made and confirmed that the hormone acts by directly stimulating the adenyl cyclase system.[15]

Distinct pharmacological actions have been demonstrated for glucagon on cardiac function, but the role of the hormone as an inotropic factor under physiological conditions remains to be established.

(iii) Electrolytes: The electrolytes composing the environment of the heart, especially sodium, potassium and calcium ions, all have distinct effects demonstrable on cardiac muscle in vitro.[16] In the intact animal, however the concentrations of these ions are maintained within relatively narrow limits and it is unlikely that they play any significant role in the

regulation of myocardial function.

(b) NEUROGENIC:

Stimulation of the sympathetic nerves to the heart produces cardio-acceleration, more powerful contractions and a reduction in diastolic dimensions.(17) In contrast to the effects of epinephrine or norepinephrine infusions, the elevation of pressure is accompanied by a tachycardia rather than by a bradycardia as in the case of the former agents. The very great increase in the tension developed by the myocardium during stimulation of sympathetic nerves to the heart may reflect some of the potential control which can be exerted through the sympathetic nervous system.

Attempts to assess the role of the parasympathetic nervous supply to the heart were made by stimulation of the vagus nerves.(11) Both left and right vagus nerves effected a reduction in heart rate on being stimulated. This was associated with a reduction in contractile force. If, however, the reduction in rate during vagal stimulation was avoided by artificial atrial pacing, no significant alteration in left ventricular performance could be shown.

Stimulation of hypothalamic and diencephalic regions in the conscious dog by means of previously implanted electrodes placed stereotactically, was found to initiate cardiac responses which closely mimicked the responses to exercise.(18) Changes in heart rate and in indices of contractility were observed that commenced with an extremely brief latent period. Such observations could be obtained by reflex mechanisms prior to the

actual initiation of exercise. The conclusion of these authors was that the traditionally accepted humoral and mechanical factors may well operate, but that higher centres were more directly responsible for the initiation of the cardiovascular responses to exercise.

B. INTRINSIC MYOCARDIAL FACTORS:

Of great interest has been the finding that in the intact animal denervation of the heart does not render the animal incapable of responding to the demands of muscular exercise.[19] The mechanisms by which the denervated heart modulates its output differ from those operating in the intact animal. Such a heart, especially after adrenalectomy, has to rely entirely on intrinsic mechanisms to control its output.

(a) Humoral Factors:

Mechanisms that may operate in primary fashion at a local myocardial level to effect an alteration in performance have been suggested. The release of neuro-transmitter substance,[20] norepinephrine and shifts of potassium ion[21] have been postulated. Monroe et al obtained convincing evidence that norepinephrine is released at a myocardial level during an increase in afterload in the isolated perfused heart preparation.[20] Sarnoff et al demonstrated a net potassium loss during increases in contractility associated with an increase in afterload and rate, but pointed out that this phenomenon may be secondary to either local pH changes associated with the increased oxygen consumption or to alterations in time relations.[21] Less time became available during diastole for the inward flux of this ion, so intimately involved with depolarization and

repolarization.

(b) Mechanochemical Factors:

(i) Synchronicity of Fibres: Evidence has been presented that the degree of synergy in muscle action is of importance in ventricular performance because of the interdigitating arrangement peculiar to cardiac muscle. Stimulation of the surface of the ventricle by an electrical impulse causes a decreased rate of pressure generation compared to a stimulus traversing the normal His-Purkinje conduction system.(22)

(ii) Frank-Starling Mechanism: It was first shown by Frank in 1895 that an increase in filling of the isolated frog's heart resulted in a more forceful contraction.(23) Starling and his co-workers related the phenomenon to an increase in fibre length as was evident by an increase in volume of their heart-lung preparation.(24)

With an increase in myocardial fibre length leading to an increase in the force of contraction, the heart has available an adaptatory mechanism enabling it to eject whatever volume is received on the input side. Serious doubt has, however, been cast on whether this relationship has any significant role in the intact animal, in contradistinction to the situation in the experimental preparations in which it was first observed and repeatedly demonstrated.(25) These arguments are based mainly on the fact that the heart has been found to decrease rather than increase in size during exercise in the face of the increased output, work and power. The Frank-Starling concept is, however, firmly grounded and consistent with the sliding-filament theory of muscle action.(26)

Recent work by Sonnenblick et al in intact unanaesthetized man, however, invoked the mechanism as actively participating in the response to exercise.(27) These authors demonstrated that tachycardia induced without exercise reduced cardiac dimensions to a larger extent than tachycardia accompanying exercise and therefore concluded that the mechanism must be operative in the exercise-response. The heart operates from an increased fibre length compared to the fibre length determined for the heart at any particular heart rate. The effect was not readily obvious by virtue of the fact that the cardiac dimensions were reduced as a result of the mechanism in operation.

(iii) Anrep Phenomenon: A second type of regulating mechanism independent of alterations in fibre length and requiring at least a few heart beats to develop, was described first by Anrep who noticed that ventricular volume at first increased but subsequently decreased following sudden elevation in aortic resistance in the heart-lung preparation.(28) This mechanism has been extensively investigated by Sarnoff and co-workers in the isolated supported heart preparation.(29) These authors have postulated that it permits the ventricle, when beating at any given rate, to eject the same stroke volume against a wide range of resistances, without requiring an increased end-diastolic fibre length. Over certain ranges it therefore acts in a manner so as to conserve the heterometric Frank-Starling mechanism for changes in stroke volume. The increase in contractility that it effects also serves to shorten the proportion of the total cardiac cycle occupied by systole. This is of particular importance when the heart rate is increased, since the diastolic interval, essential for both ventricular

filling and coronary artery perfusion, is maintained at maximum.

(iv) The Bowditch Effect: Bowditch first observed that an increase in stimulation frequency is associated with an increase in twitch tension in the isolated frog heart.[30] Hajdu has investigated this phenomenon extensively and correlates the alteration in twitch tension with a shift of intracellular potassium ion to the extracellular space. The decreased intracellular potassium concentration, to a certain limit when sustained contracture ensues, is associated with an increase in contractile force. The cause of the potassium shift has been postulated to be related to either changes in pH or to the relatively decreased time available for shift of the potassium in an intracellular direction because of the shortened diastolic interval. In the mammalian heart Sarnoff et al have been able to demonstrate not only the phenomenon of the staircase[29] but also the potassium shifts.[21] Manifestations of the same mechanism are also found in postextrasystolic potentiation[31] and after sustained postextrasystolic stimulation e.g. paired electrical pacing[32] both of which situations are associated with an increase in contractility.

The operative mechanism remains to be precisely defined; it has been suggested, in addition to the views of Hajdu[30] outlined above, that local release of norepinephrine[33] may have a role in the mediation of the phenomenon. Recent investigations into the changes of calcium ion flux across the cardiac muscle membrane by Langer and Brady[34] have strongly suggested that this ion and membrane permeability to it, are important factors in determining inotropic phenomena.

According to these investigators, increased frequency of contraction initiates a process of augmented calcium turnover with influx initially exceeding efflux. A distinct calcium transport system appears to be operative; this contention was supported by the work of Grossman and Furchgott who, at various calcium concentrations and stimulation frequencies in guinea-pig atria, demonstrated a positive relationship between calcium exchange and contractility.(35)

3. MYOCARDIAL CONTRACTILITY AND ITS ASSESSMENT IN THE INTACT ANIMAL.

A. PHYSIOLOGY OF CONTRACTION IN ISOLATED MUSCLE:

The performance of the heart has traditionally been described in terms of relations between the end-diastolic volume, or filling pressure, of the ventricles and their output, either of volume of blood or work performed.(36) While these measurements provide considerable insight into the level of cardiac performance, studies on the mechanics of isolated muscle and heart preparations have suggested that the contractile state of cardiac muscle, like that of skeletal muscle, can be characterized best in terms of the relation between instantaneous force, velocity of shortening and fibre length.(37)

(i) Muscle Structure:

According to the Three-Component theory of muscle morphology the muscle, including cardiac muscle, behaves as if it were composed of three functional mechanical components.(38)

(a) the active contractile element which can shorten and develop tension.

(b) a passive series elastic component, mechanically in series with the contractile element.

(c) a passive elastic component, mechanically in parallel with the contractile element, the parallel elastic component.

These are illustrated conceptually in Fig.1 according to Jewell and Wilkie.

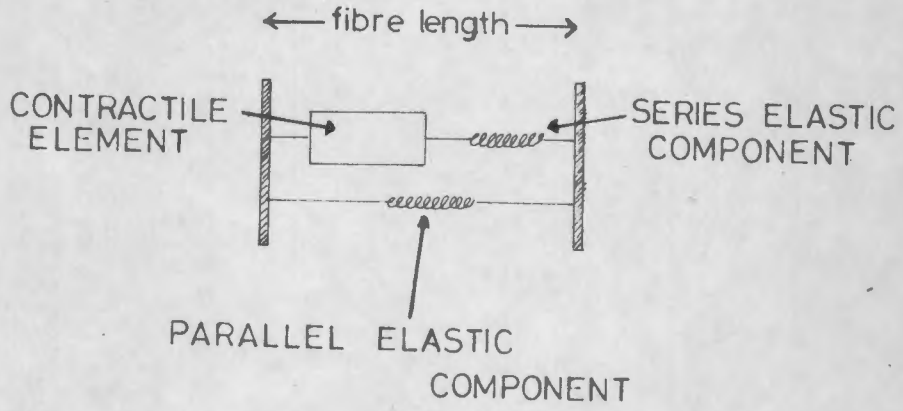


FIG.1

(ii) Length-force-velocity relationships in striated muscle:

The length and tension changes in the muscle, as a whole, depend on the interactions among the three elements outlined previously. A complete description of the contractile state of a muscle, whether skeletal or cardiac, can be achieved by the use of four variables, all of which may, however, change simultaneously during the course of a single contraction. These variables are:

(i) the tension in the muscle.

(ii) the velocity of shortening of the contractile element.

(iii) the length of the fibre.

(iv) time relationships of tension development after activation.

The interactions of these factors have been shown to obey certain constant mathematical relationships which can be expressed in the form of curves, first described by A.V. Hill on the basis of heat production, but more recently in terms of

actually measured velocity and shortening.[39] The mechanical constancy of such relationships were emphasized by Ritchie and Wilkie who were able to predict the responses of muscle under varying states of fibre length and load, by demonstrating the close correlation between predicted and observed responses.[40] The curves required by these authors to fully describe the action of muscle were:

(i) the force-extension curve of the series elastic component.

(ii) the curve relating the intensity of the active state of the fully active muscle to muscle length.

(iii) the force-velocity curve of the fully active muscle.

(iv) the curve describing the change in the intensity of the active state with time after a single stimulus.

Since the second, third and fourth curves relate the fundamental behaviour of the contractile element, they may in fact be used to characterize the contractile state of the muscle.

(iii) Isometric Length-Force-Velocity Relationships in Isolated Cardiac Muscle.

These curves were described for skeletal muscle which could be tetanized in the fully active state and plateaux of force generation obtained. The identical relationships were shown to apply to cardiac muscle which, even though it cannot be tetanized, permits points obtained from successive contractions and representing a series of instantaneous curves, to be utilized instead. Using isolated cat papillary muscles, Abbott and Mommaerts[41] and Sonnenblick[42] have shown that

the relation between the total load lifted by an isotonically contracting after-loaded muscle and the initial velocity of isotonic shortening is a curve identical to that for skeletal muscle. This is shown in Fig.2 (after Sonnenblick).[42]

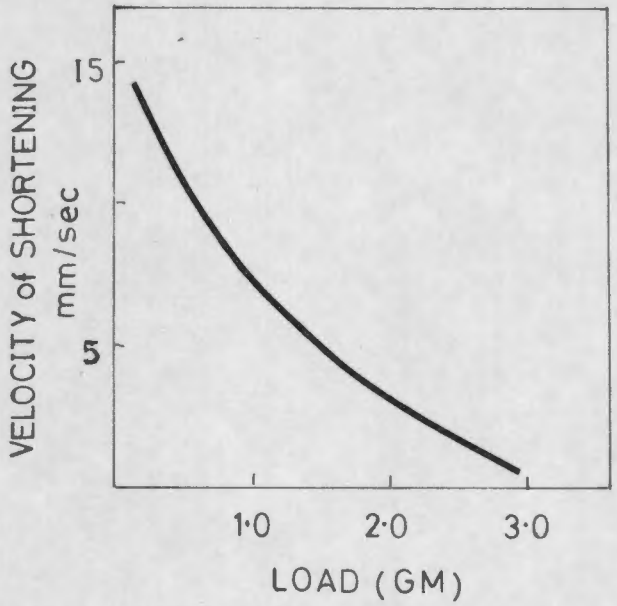


FIG. 2

In the isometric twitch, certain characteristics of cardiac muscle are evident:

(1) As the initial length of the muscle fibre is increased, the tension developed and the rate of tension development both increase, so that the interval from the onset of contraction to the peak of tension development remains constant, provided the frequency of contraction, temperature and ionic environment remain the same. Fig.3, redrawn from Sonnenblick[42] illustrates this aspect.

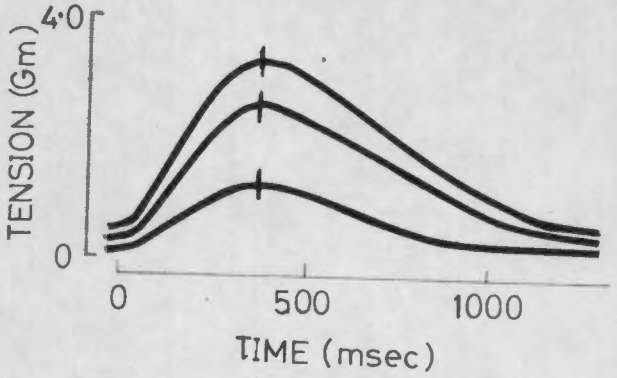


FIG.3

(2) When the initial muscle length is increased during the period of tension recording, both resting tension and developed tension (height of tension curve) are increased. The behaviour of resting tension and developed tension on increase in initial length occurs according to the relationship shown in Fig.4, redrawn from Sonnenblick.(42)

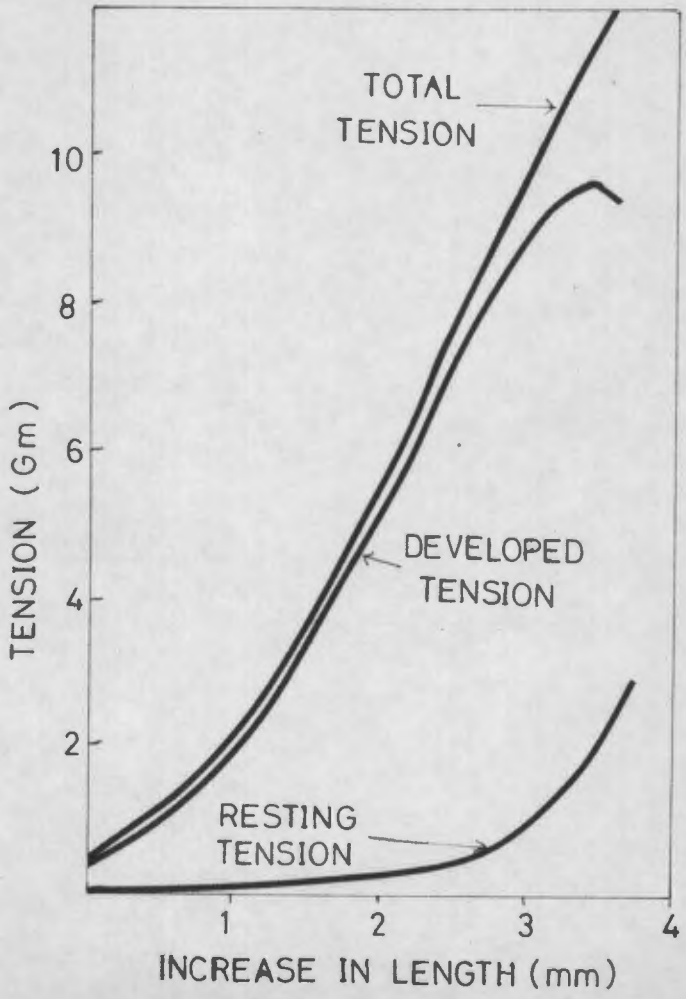


FIG.4

(3) By increasing the frequency of contraction, both the rate of tension development and the developed tension are increased, illustrated in Fig.5, redrawn from Sonnenblick.[42]

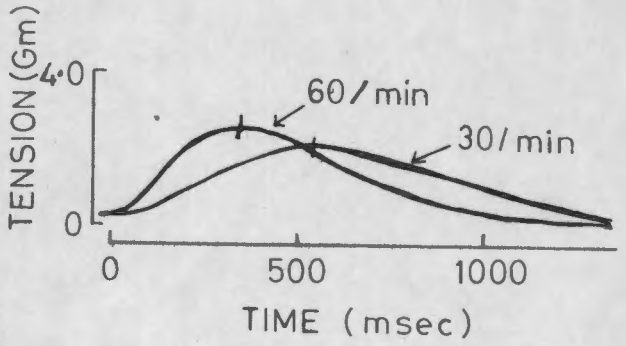


FIG. 5

(4) Inotropic Interventions:

The addition of inotropic agents e.g. norepinephrine or additional calcium ions, bring about certain specific alterations in the relationships of force, velocity and time.

(i) Increase in calcium concentration results in an increase in the rate of tension development and the peak developed tension, with minimal reduction in the time-to-peak-tension period.

(ii) Addition of norepinephrine results in an increase in the rate of tension development and peak developed tension with also a substantial shortening of the time-to-peak-tension period.

(iv) Isotonic Force-Velocity Relationships in Cardiac Muscle:

The same relationships were investigated in isotonically-contracting cardiac muscle.[37,42] A small adjustable preload was provided and the preloaded length-tension relationship obtained. The muscle was then allowed to develop tension against an afterload and overall shortening occurred once the afterload was lifted. The initial velocity of shortening on

lifting the afterload was plotted against the total load; a curve similar again to that of skeletal muscle was obtained. As the afterload was increased, the muscle developed increased force to meet this load, following which shortening occurred. As the afterload increased, the initial velocity of isotonic shortening and the extent of shortening progressively decreased, as is shown in Fig.6 - redrawn from Sonnenblick.(42)

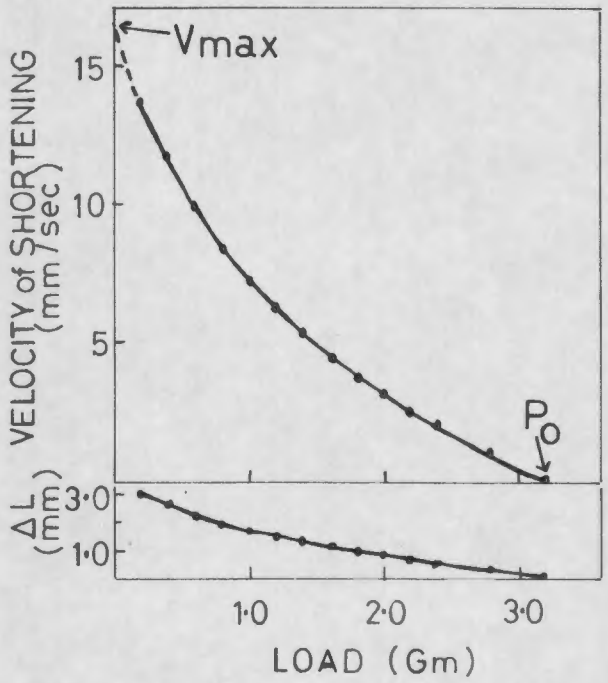


FIG.6

The curve relating velocity of shortening to load is once again identical to the curves seen for skeletal muscle.(39,40)

The theoretical point, with no load on the muscle, or conversely, totally unopposed force generation, when velocity of shortening by extrapolation would be maximal, is labelled as "Vmax". (c.f. Fig.6). Since in accordance with the resting length-tension relation of the papillary muscle, a significant preload is necessary to establish the initial length that will produce such a contraction, Vmax cannot be directly determined, but is obtainable only by extrapolation.

Similarly if the afterload is increased to the point at which no shortening occurs and the contraction occurs isometrically, the isometric twitch-tension is obtained, "Po". This point is also indicated in Fig.6.

(a) Effect of varying initial length on isotonic force-velocity relationships.

If the initial length of the muscle is increased by increasing the preload, with each increase in initial length a new curve is obtained, yielding a series of force-velocity curves which, when extrapolated to zero load, produce convergence to a common Vmax; this is illustrated in Fig.7 - redrawn from Sonnenblick.[42] In this figure each individual curve represents the force-velocity relationship for a specific initial muscle length.

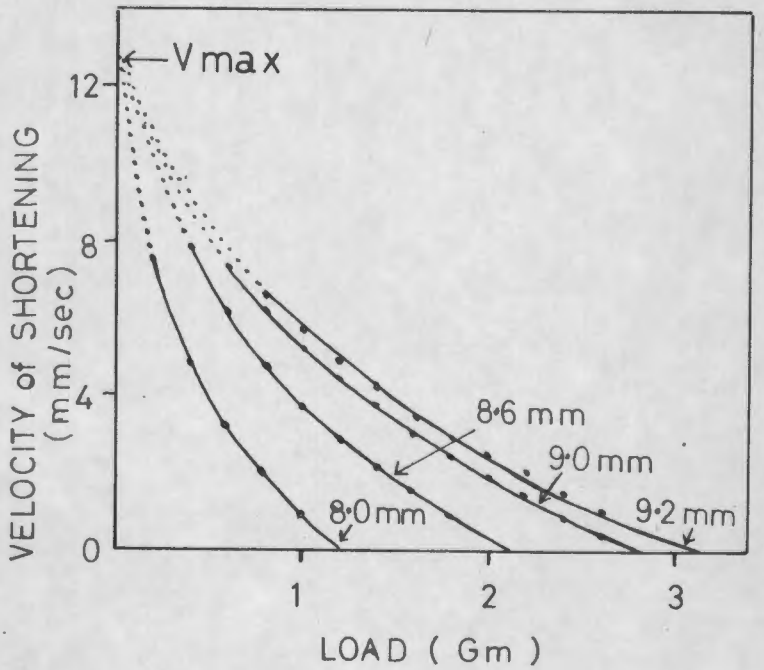


FIG.7

If the external work performed by the muscle (i.e. total load X distance of shortening) and its power (total load X velocity of shortening) are calculated, the relationships are as represented in Fig.8, redrawn from Sonnenblick.[42]

At any given initial muscle length, with constant frequency of contraction and chemical environment, the work and power of the muscle become necessary functions of the load.

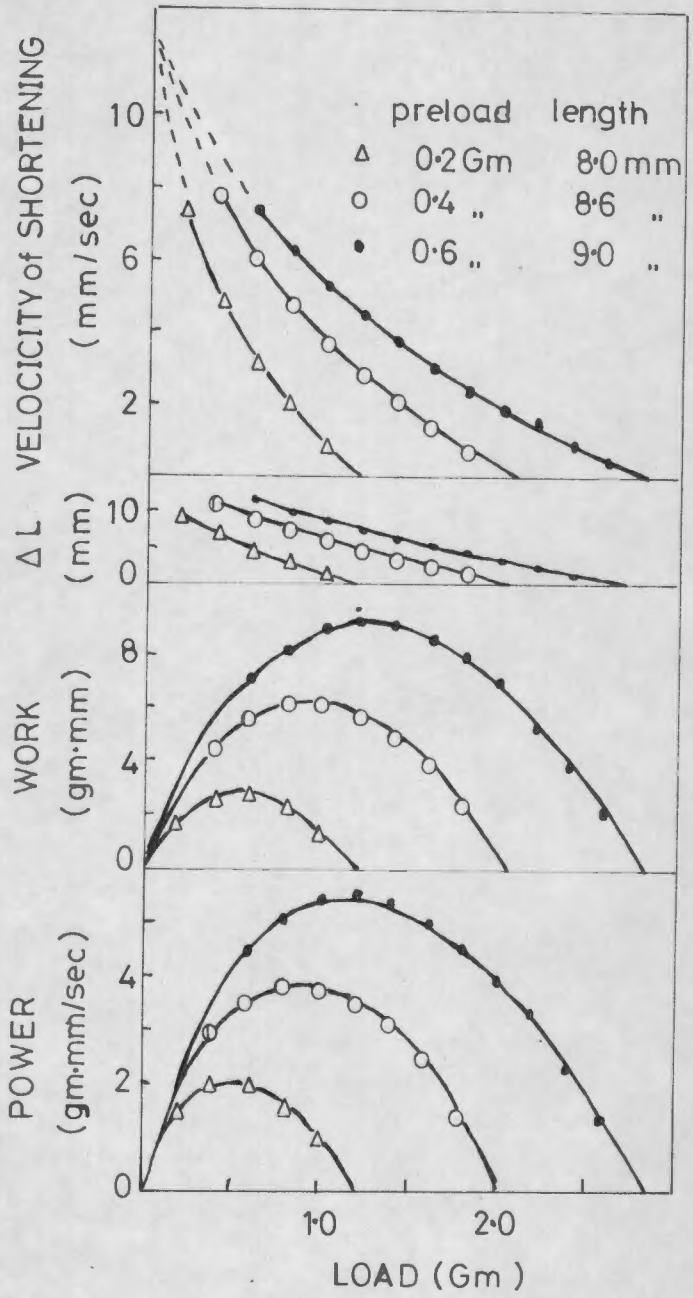


FIG. 8

As initial muscle length is increased, the maximal work the muscle would perform, as well as the load at which the maximal work is performed, are both increased. At any one load, increasing initial muscle length, increases the work accomplished

and the velocity of shortening; hence the power is increased as well.

(b) <u>Effect of inotropic interventions and changes of rate on force-velocity relations.</u>

If pre-load, initial length and afterload are held constant, the rate of tension development (dT/dt) and the velocity of shortening (dl/dt) could be changed by varying the frequency of stimulation, by changing the environmental calcium concentration or by the addition of an inotropic agent e.g. norepinephrine. It can be shown that such inotropic interventions produce their effects without alteration in the elastic components. Such effects are shown in Fig.9 (redrawn from Sonnenblick.[42])

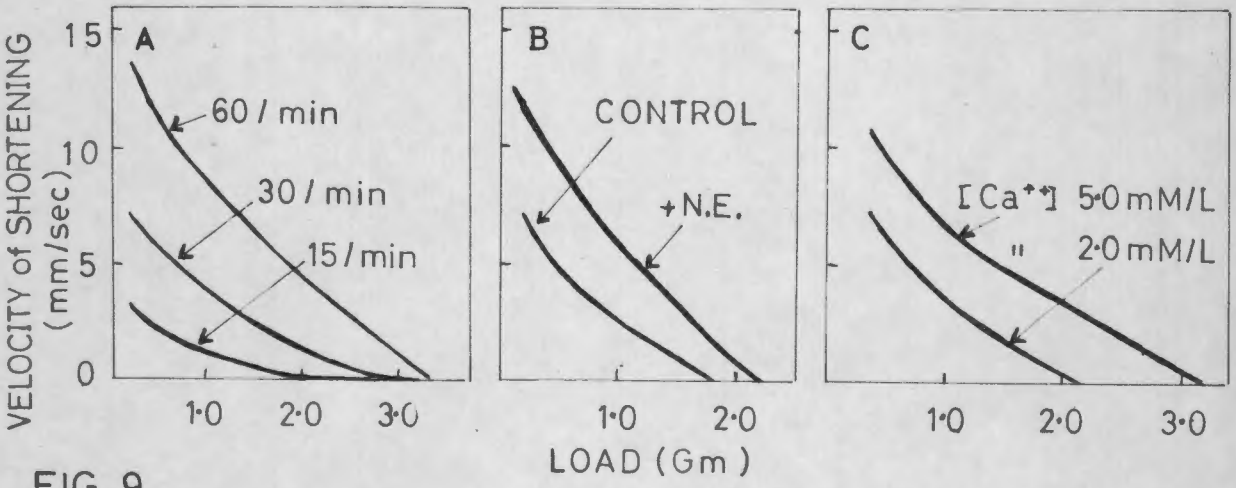


FIG.9

In each instance the initial muscle length is held constant. In panel A it can be seen that as frequency of contraction is increased there is an increase in Vmax, with little or no increase in Po. When norepinephrine is added an increase in both Vmax and Po was observed (Panel B). When $[Ca^{++}]$ is increased, there is also an increase in both Vmax and Po with a parallel shift in the curve (Panel C).

(v) Force-velocity relationships and intrinsic contractility:

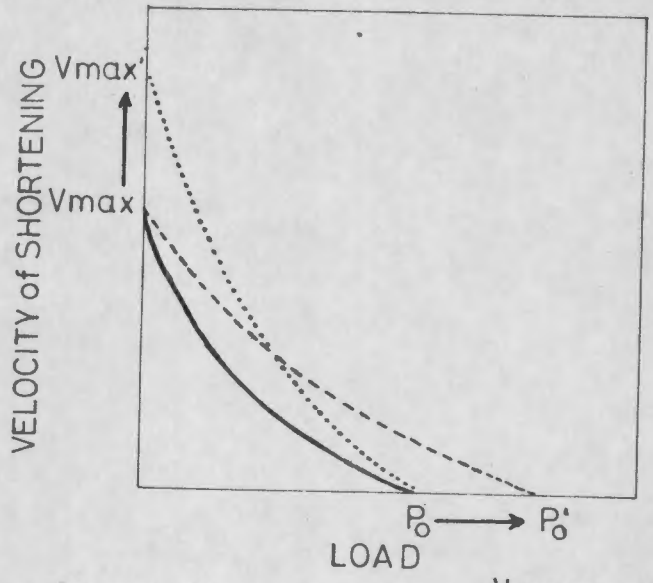
From the foregoing, it becomes evident that cardiac muscle will readily, under the influence of inotropic stimuli, alter its relationship between Po and Vmax i.e. its force-velocity relationships. It is this readiness with which cardiac muscle will alter its force-velocity relations and hence its contractile state, which provides a fundamental functional distinction between cardiac muscle and skeletal muscle. In changing Vmax, an inotropic intervention induces a change in the basic state of the muscle and an altered state of contractility is engendered. Inotropic actions are accompanied by shortening of the duration of contraction, hence changes in the rate of force development at any one muscle length should reflect alterations in the force-velocity relation alone without necessary alteration in length-tension relations.

Two important generalities about cardiac muscle therefore become apparent:

(i) an increase in muscle length induces an increase in the force of contraction (as in skeletal muscle). With increasing fibre length, the increase in force (Po) is engendered without a change in Vmax. Vmax therefore remains to define and quantify the basic state of the muscle.

(ii) At any one muscle length increasing frequency of contraction or alteration in chemical environment may readily alter the rate of force development or the peak force developed, without prolongation of the duration of contraction. A change in Vmax will therefore have occurred.

A summary of these principles is provided in Fig.10 - redrawn from Sonnenblick.(37)



	Vmax	P_o
INCREASING LENGTH	–	↑↑
INCREASING RATE	↑	↑or –
NOREPINEPHRINE	↑↑	↑

FIG. 10

In changing Vmax an inotropic intervention induces a change in the basic state of the muscle and hence changes its contractility.

(vi) <u>Definition of Contractility</u>:

After these considerations one may define contractility of cardiac muscle as follows:

"Changes in myocardial contractility may be defined as changes in the performance of the muscle that arise from alterations in the relationship, during the active state, among force, velocity, fibre length and the time-course after excitation".

Using these terms, changes in the performance of the heart resulting from changes in the fundamental properties of the contractile machinery of the muscle are said to represent changes in myocardial contractility; changes in performance

arising secondarily from changes in physical conditions outside the contractile elements e.g. increase in load or fibre length, are not.

B. PHYSIOLOGY OF THE MYOCARDIUM IN THE INTACT HEART.

Preparations used in the study of isolated cardiac muscle are necessarily deprived of blood and nervous supply and can therefore hardly be considered as metabolically normal. Moreover the responses of these electrically driven preparations need not necessarily reflect the behaviour of the rhythmically beating heart.

Aside from the identity of the contractile machinery, certain mechanical parallels may be noted between the operation of the papillary muscle and the intact heart. As a gross approximation, the heart functions as an afterloaded muscle i.e. the heart has its initial fibre length established by a small preload (ventricular end-diastolic pressure or effective filling pressure); with activation the myocardium develops a force equal to the afterload (aortic pressure) after which shortening of the muscle occurs with ejection.

(1) Length-Tension Relationships in the Intact Heart.

Frank, using the isolated frog's heart, demonstrated that the height of isometric tension generated by the left ventricle depends on the initial filling of the ventricle.(23) He interpreted his findings, though, as indicating that it is the initial tension in the muscle, rather than fibre length which acted as the factor determining the force of the subsequent contraction. Frank also succeeded in showing that the output of the ventricle increased as its filling was increased.

He did not, however, measure the volume of the ventricle and again interpreted his findings as pointing to the initial tension in the muscle as the determinant of the increased output.

Using the dog's heart-lung preparation, Patterson, Piper and Starling demonstrated that it was by increasing its volume and hence the length of its fibres that the heart increased its force of contraction and stroke output.(43). These workers observed that the tension as assessed by intraventricular pressure recording, need not change at all in the presence of large increases in volume on increasing the output of their preparation. This led them to the conclusion that, "the output of the heart is a function of its filling, the energy of its contraction depends on the state of dilatation of the heart's cavities" which forms the basis of Starling's Law of the Heart.(24)

Subsequently others have concluded that it is the intraventricular tension rather than fibre length(44) or both factors(45), end-diastolic fibre length and initial tension, that are responsible for the characteristics of the subsequent contraction.

In a definitive study, Braunwald et al(46) measured simultaneously left ventricular circumference, using a mercury-filled resistance gauge, and intraventricular pressure. The relationship between left ventricular end-diastolic pressure and end-diastolic circumference was examined under conditions of varying output and resistance to outflow. These investigators found that the relationship between left ventricular end-diastolic pressure and end-diastolic volume remained constant when both aortic pressure and stroke output were varied over wide ranges. An increase in myocardial contractility was

accompanied by increased cardiac output while maintaining the same end-diastolic pressure and volume. At the end of their experiments, depression of myocardial function was always indicated by an increase in end-diastolic circumference at any given end-diastolic pressure. These observations lend support to the view that in the intact heart it is the circumference rather than the intraventricular pressure that influences the force generated by the subsequent beat, as originally proposed by Patterson, Piper and Starling.

Linden and Mitchell[47] came to the same conclusions in their study of the relation between left ventricular diastolic pressure and myocardial segment length. These authors defined a curvilinear relationship between these two variables in the intact heart, as illustrated in Fig.11 (from Linden and Mitchell).

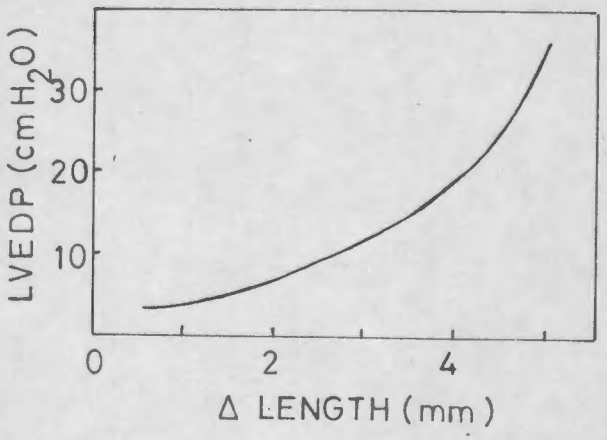


FIG.11

Two portions of the curve are apparent, one in which during low ventricular pressure, a small increase in pressure is accompanied by a large increment in myocardial segment length and one in which at high end-diastolic pressures a substantial increase in pressure is required to elicit a small increase in segment length.

The clinical implications of these relationships have been discussed by Braunwald.[48]

Starling's Curve:

The relationship between end-diastolic pressure, end-diastolic volume and the output of the heart was considered by Frank[23] and formed the basis of Starling's Linacre Lecture on the Law of the Heart.[24] A curve was described along which the heart behaves in terms of output as the inflow is varied. The curve had three portions; an ascending portion, an apical flat portion and a descending portion, interpreted to indicate a state of failure of the heart to meet its demands. This curve was based on work performed in the heart-lung preparation and serious doubt was cast on whether these relationships applied to the function of the intact animal's heart.[25]

Sarnoff and Berglund[36] using the intact dog, made a meticulous study of the applicability of Starling's Law of the Heart to their preparation. These authors examined the relationships between filling pressure and ventricular stroke work rather than volume, under a wide range of physiological circumstances. Sarnoff's curves were obtained by plotting the ventricular stroke work (in gram meters per stroke) against the mean atrial pressure on the same side of the heart. A typical example is shown in Fig.12 - redrawn from Sarnoff and Berglund.[36] This curve showed an initial steep rise at low filling pressure and flattened off to a plateau with little or no decline even at high filling pressures. These authors could never demonstrate such a descending limb i.e. a fall in stroke work at high filling pressures in the normal heart with an intact pericardium. It could be demonstrated, however:

(1) when left main coronary artery flow was compromised.

(2) in the presence of severe anaemia.

(3) when the pericardium was widely opened.

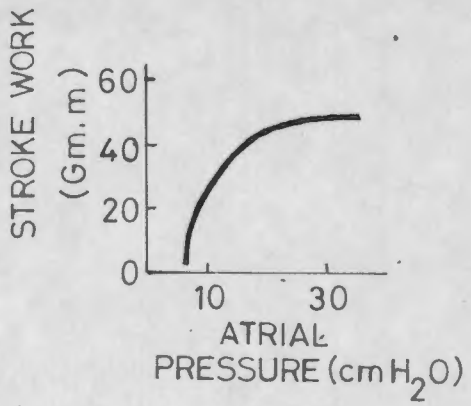


FIG.12

These authors stressed furthermore the importance of using stroke work which takes into consideration the pressure against which the output is delivered rather than output alone. They came to the conclusion that a single Starling's curve cannot always satisfactorily describe the function of the heart, but that for a given heart there is a series or family of Starling curves, with the heart having the capacity to move from one curve to another depending on the functional state of its control Starling curve and begin to function on a new higher curve.

On the basis of these observations Sarnoff has defined myocardial contractility as follows.(49)

"When from any given end-diastolic pressure or fibre length, the ventricle produces more external work or more external stroke power (stroke work per systolic second) an increase in ventricular contractility is said to have taken place".

Implicit in this definition is an increased rate of development of tension when contractility increases. Specifically excluded is any increased work that may be performed as the result of an increased afterload from the same end-diastolic length, since the rate of development of tension is not increased

under such circumstances.

In a further series of experiments investigating the role of the central nervous system on ventricular performance, Sarnoff et al, demonstrated that the position of the heart on a particular Starling curve was determined by the level of end-diastolic filling of the left ventricle, whereas the particular Starling curve the heart was operating on, was determined by the degree of sympathetic or para-sympathetic stimulation.(50) These authors demonstrated that the sympathetic system affected ventricular contractility directly, but that the effect of vagal stimulation on the ventricle was secondary to its effect on atrial activity. Sarnoff and Mitchell integrated their findings(51) and concluded that the use of the left ventricular end-diastolic pressure as a determinant of left ventricular stroke work or power in the construction of left ventricular function curves would appear to be quite valid. These investigations countered to a large extent the objections raised by Rushmer(52) who felt that in the intact animal the basic muscle physiology applicable to isolated preparations was so obscured by nervous and humoral factors that the basic principles were never called into action. In later work the latter author(53) has demonstrated the validity of the assumptions governing the pressure-circumference relations in the left ventricle in the intact unanaesthetized animal.

(c) <u>The Role of the Afterload</u>:

In the intact animal the role of the changing afterload or arterial pressure on length-tension relationships arises as an important question not applicable to the isolated muscle preparations where this remains constant. This aspect was

investigated by Sonnenblick and Downing.[54] These workers, using the cat heart preparation, evaluated stroke work as a function of LVEDP at different controlled levels of aortic pressure and showed that at any given LVEDP stroke work was very much dependent upon aortic pressure. Over a wide range of aortic pressures (75 - 120 mm Hg mean aortic pressure), stroke volume was maintained constant. Only when mean arterial pressure was raised to more than 150 mm Hg did a decrease in stroke volume occur at the same LVEDP. Their conclusion was therefore that stroke work (product of stroke volume and mean aortic pressure) is determined in the intact heart mainly by the contractile state and the aortic pressure. An increase in contractility induced by norepinephrine was consistently accompanied by an increase in stroke volume at any given LVEDP. They also suggest that alterations in contractility may be better described in terms of the relation between stroke volume and LVEDP rather than between stroke work and LVEDP, thus excluding the limitations imposed by a changing afterload. The same limitations also apply when using stroke power (stroke work/duration of ejection) to define contractility. The relation of ventricular ejection rate (stroke volume/duration of ejection) to LVEDP was suggested as a useful measure of contractility by adding the dimension of time without the limitations of the afterload.

(d) Heterometric and Homeometric Autoregulation:

Taking into consideration these principles, the stroke work of a ventricle depends on two separate intrinsic mechanisms at any given state of contractility. Firstly, the ventricular work may be increased at a constant arterial pressure by augmentation of stroke volume. This involves the Frank-Starling

principle and involves an increase in end-diastolic ventricular volume. Secondly, if the stroke volume is maintained constant, the arterial pressure and consequently the work of the ventricle may be increased over a wide range with very little alteration in LVEDP. This is in accordance with the afterload principle and allows for large changes in ventricular work without impinging on the reserves provided by the Frank-Starling principle. These two phenomena have been termed heterometric and homeometric forms of autoregulation respectively by Sarnoff.[29,49] Homeometric autoregulation has important implications:

(i) it allows the ventricle beating at any given rate to eject the same stroke volume against a wide range of resistances without requiring an increased end-diastolic fibre length with its attendant mechanical disadvantage. In a fashion it acts to ensure heterometric autoregulation over certain ranges of function.

(ii) the increases in contractility, especially that aspect of it exhibited as a more rapidly developed ventricular pressure, shortens the proportion of the total cardiac cycle that is occupied by systole which is, of course, of great advantage when heart rate is increased. Diastolic interval with its important implications for ventricular filling and myocardial perfusion is maintained as long as possible.

The application of these principles in the function of the human heart has recently been outlined by Braunwald.[55]

(2) FORCE-VELOCITY RELATIONSHIPS IN THE INTACT HEART.

The work of Frank in the frog's heart[23] and Peserico[56] in the tortoise heart, suggested an inverse relation between

the rate of ventricular contraction and the resistance against which the ventricle ejected. Fry et al[57] and Levine and Britman[58] have investigated the force-velocity relationships in the intact heart more fundamentally, attempting to formulate these relationships in terms of the rate of shortening of the contractile elements themselves and the intramyocardial force generated. These investigators have demonstrated conclusively that the force-velocity relationship existing in isolated cardiac muscle applies also to the contractile elements of the intact metabolically-supported spontaneously-beating mammalian heart.

However, aortic and ventricular pressures do not remain constant throughout the cycle, particularly during ejection and intraventricular pressure cannot be directly equated with intramyocardial force since, in accordance with the La Place formulation, the relation is greatly dependent on ventricular volume, which changes once ejection commences. These investigators have therefore attempted to establish the relationships between intramyocardial force, velocity of shortening and fibre length at any instant in the cycle. A series of instantaneous observations are then considered.

A.V. Hill's model of the components of skeletal muscle is therefore adapted for cardiac muscle in the intact heart as shown schematically in Fig.13. It consists of a circumferential arrangement of contractile elements attached to one another by a series of relatively stiff springs which represent the series elastic components of the heart. The elasticity represented by the other spring, running parallel to the contractile elements, represents the parallel elastic component.

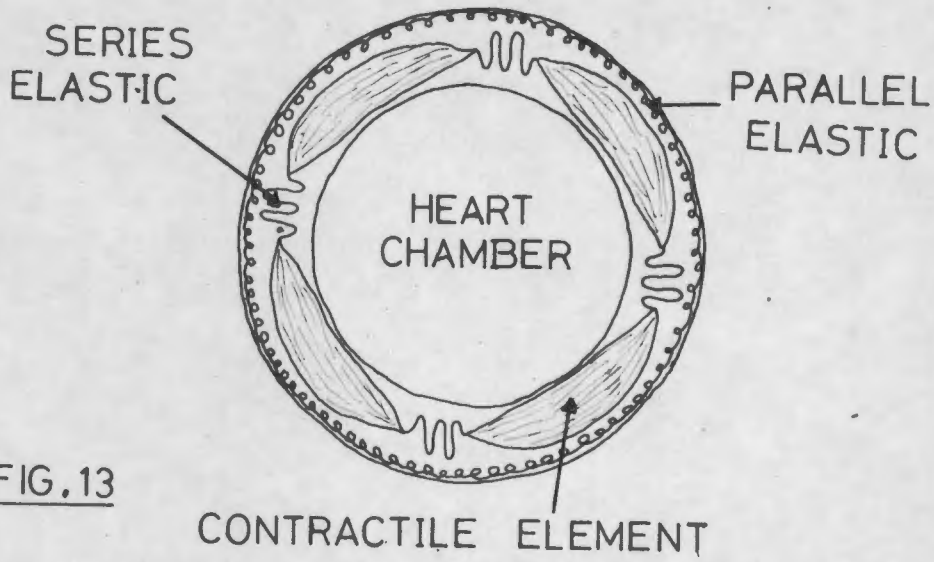


FIG. 13

One would therefore visualize systolic ejection as the result of a group of contractile elements which are shortening and applying tangential tension through a series of springs to a hollow cavity. The presence of these springs means that the shortening velocity of the contractile elements will not be the same as the shortening velocity of the muscle as a whole.

The availability of electromagnetic flowmeters placed around the root of the aorta, electrical recording calipers and high-fidelity pressure transducers has made it possible to obtain simultaneous observations of pressure, volume and flow at selected points during the cardiac cycle.

(i) Obtaining "Force" in the Intact Heart:

The ventricle is considered to be a sphere. The instantaneous systolic volume can be obtained by subtracting from the end-diastolic volume the quantity of blood ejected up to the point of measurement, this being obtained from the flowmeter tracings. From this, the instantaneous radius can be calculated by solving for r in the formula:

$$v = \frac{4}{3}\pi r^3$$

(where v is the instantaneous systolic volume).

The total tension (T) developed by the myocardium at its equator can be obtained from the formula.

$$T = P \cdot \pi r^2$$

(where P is the intraventricular pressure in grams/cm^2)
This is then normalized according to the formula.

$$T' = \frac{Pr'}{2s}$$

(where T' is tension per unit length of circumference per unit of wall thickness).

P = intraventricular pressure in gm/cm^2

r' = midwall thickness in cm

s = instantaneous ventricular wall thickness in cm

This tension, T, represents the <u>force</u> in Hill's force-velocity relationship.

(ii) <u>Obtaining Velocity of Shortening in the Intact Heart</u>:

The instantaneous rate of shortening of the circumferential fibres comprising the inner equator of the ventricle (Vcf) can be calculated from the flow rate, obtained by differentiation of the flowmeter tracing.

$$dv/dt = 4\pi r^2 dr/dt$$

(dv/dt = aortic flow rate)

and since $Vcf = 2\pi dr/dt$

by substitution

$$Vcf = \frac{dv/dt}{2r^2}$$

i.e. $$\frac{\text{aortic flow rate}}{2r^2}$$

To calculate the shortening velocity of the contractile elements themselves it is necessary first to derive the rate of lengthen-

ing of the series elastic component.

The rate of tension development (dT/dt) is related to both the stiffness of the series elastic component (dT/dl) and the rate at which the series elastic component lengthens (dl/dt).

This relation $dT/dt = (dT/dl)(dl/dt)$

may be arranged $dl/dt = \frac{dT/dt}{dT/dl}$

The stiffness of the SEC, dT/dl can be calculated from a value of T obtained from a coefficient derived from isolated cardiac muscle and involves the assumption that its modulus of elasticity remains constant during the entire contraction.

dT/dt can be calculated from the measured dp/dt since

$$T = \frac{Pr}{2}$$

where T = tension per unit length of circumference

P = intraventricular pressure in gm/cm^2

$\therefore \quad dT/dt = \frac{1}{2}r \cdot dp/dt + p(dv/dt)$

Since $V_{cf} = -2\pi\, dv/dt$, by substitution

$$dT/dt = \frac{r}{2} \cdot dp/dt - p \cdot \frac{V_{cf}}{4\pi}$$

This supplies one with dl/dt (per cm of circumference) i.e. the rate of lengthening of the SEC, which occurs as the rate of shortening of the contractile element. This value dl/dt multiplied by the circumference of the ventricle can be considered equal to the Vce, the "velocity" of the force-velocity relationship. During ventricular ejection dl/dt is added to Vcf to obtain Vce to compensate for the change in volume that occurs.

When Vcf and Vce were plotted against myocardial wall tension at isovolumic points in beats subjected to a range of afterloads, an inverse relation between tension and velocity

could be demonstrated by both groups of authors. In addition, it was shown that this relation between force and velocity, and its extrapolations to Vmax and Po were markedly altered by norepinephrine and in the opposite direction by acute heart failure or the administration of barbiturates.
The relationships in the intact heart may thus be summarized:

(1) there is a reciprocal relationship between the tension in the muscle and the velocity with which it is shortening at any instantaneous volume.
(2) for any instantaneous tension the velocity of shortening increases with heart volume.

Fry et al made the observation that the tension-velocity relationship in the intact heart is greatly dependent on fibre length and suggested that with reference to the cardiac muscle, it is more appropriate to refer to its length-tension-velocity relationships.

These methods, although considerably more sophisticated than any others previously used, still involve several assumptions, mainly concerning the effects of alterations in volume of the ventricle during ejection. In order to obviate these, Ross et al resorted to the definition of the force-velocity relation in single isovolumic beats, obtained by rapidly occluding the aortic root by means of a pressure-driven balloon.[59] They showed that such determinations of force-velocity curves could provide insight not only into the level of contractility, but also provided a means whereby the influence of changes in resting fibre-length on the force-velocity relation could be analysed. Similar observations have recently been reported in the conscious dog by Taylor et al.[60]

Using radio-opaque markers sewn on to the ventricle and high speed radiophotography, Sonnenblick et al have also established the application of the force-velocity relation to the human heart.[27,61]

(3) Active State in Muscle and Force-Velocity Relation:

Ross et al[59] observed that when Vce was plotted against time during single contractions, a measurable delay in the achievement of maximum Vce always occurred. These findings agreed with those of Levine et al[62] who found that the ventricle is mechanically fully activated with its active state close to maximal within 30-50 msecs after the onset of pressure rise in the intact heart. Ross et al[59] also demonstrated by comparison of isovolumic and isotonic beats, a secondary rise in Vce at the onset of ejection in the isotonic beats. Vce and particularly its maximum rate of shortening varies therefore even within the isometric phase of ventricular contraction. Angelakos et al[63] made the same observation using a sensitive intraventricular micromanometer.

These changes in active state were not observed by Levine and Britman[58] using the measurement of blood flow at the root of the aorta as an index of the rate of contraction. The velocity of blood flow at the root of the aorta may therefore not accurately reflect events in the myocardium. The suggestion becomes evident that the measurement of intraventricular pressure remains the most faithful measure of myocardial mechanics available. The reason for these alterations in active state are not clear. It has been suggested that these may be entirely the result of alterations in compliance occurring with realignment of fibres during contraction[57] and may

not be true alterations in contractile element action. Sonnenblick, however, suggested as a result of observations in cat papillary muscles, that in cardiac muscle, for a given contractile state, once shortening has commenced, the instantaneous velocity of shortening becomes a function of instantaneous muscle length, essentially independent of the point at which shortening began.(64) Inotropic interventions accelerate the onset of the maximal active state, increase its intensity, shorten its duration and hasten its decline.(65) Similar conclusions are drawn by Brady(66) who points out that the force of the contractile element must precede externally manifested tension, at least by a small segment of time, but at present the isometric contraction itself appears to be the best available index of the time course of the active state in heart muscle. These concepts have recently been synthesized into a three-dimensional model describing the interrelationship of these various factors.(67,68)

C. DETERMINATION OF CONTRACTILE STATE IN THE HEART OF THE INTACT ANIMAL.

Highly sophisticated apparatus and techniques were required to establish the principles previously outlined and to demonstrate that the same fundamental relationships between force, velocity of shortening and fibre length which obtain in isolated cardiac muscle are also applicable to the functional contractile elements of the intact spontaneously beating heart. Extensive surgical manipulation, anaesthesia and open-chested preparations were often necessary. Such measures are obviously not practical or even possible in the study of the fully intact conscious animal or subject. It therefore remains to select aspects of these techniques that could be applicable to the study of the intact animal.

(a) Pressure Measurement in the Isometric Phase of Ventricular Contraction.

It has already been indicated that the measurement of intraventricular pressure[59,65] remains the most faithful measure of myocardial mechanics available. Likewise it has also been stressed by Brady that analysis of the isometric cardiac contractions gives the best insight into the time course of the active state in heart muscle.[66] It remains therefore to investigate the feasibility of utilizing the measurement of pressure during the isometric phase of ventricular contraction as an index of the contractile state of the ventricular muscle, since measurement of intraventricular pressure, although presenting certain problems pertaining to catheter systems, remains a simple and readily adaptable procedure. One is obliged to assess, however, to what degree accuracy has to be sacrificed for simplicity and applicability.

The value of the rate of development of ventricular pressure as an index of performance capacity was first commented on by Frank who noted in his experiments on the isometric curve of contraction of the frog's heart that the gradient of the slope of the ascending portion of ventricular pressure is determined by the degree of ventricular filling.(23) He also defined the segment of the maximal velocity of shortening by stating that the maximal velocity is prior to the peak pressure being reached. Wiggers observed in the dog's heart that as LVEDP is increased, the height of pressure and the slope of the isometric curve both become greater.(69) He also demonstrated that when adrenaline was administered to his preparation an increase in the height of pressure and the gradient of the slope during the isometric phase of ventricular pressure occurred without any concomitant rise in LVEDP.(45) Wiggers not only defined the fact that the isometric period showed a steeper rise, but also that it occupied a shorter period of time; i.e. max dp/dt was increased.

(b) Determinants of LVmax dp/dt.

The haemodynamic determinants of the maximal rate of rise of pressure (max dp/dt) have been investigated in detail by several groups of workers.(70,71) The findings of Wallace and his co-workers(71) can be summarized as follows:-

(1) if the heart rate is increased, (their range was 80 to 220 beats/minute), LVmax dp/dt increases in linear fashion. Aortic pressure and stroke volume were kept constant.

(2) LVmax dp/dt increases linearly with left ventricular end-diastolic pressure (LVEDP), achieved by increas-

ing stroke volume at constant aortic pressure.

(3) if aortic pressure is increased while maintaining rate and LVEDP constant, LVmax dp/dt increases linearly in the range between 60 and 140 mm Hg mean pressure.

(4) if ventricular activation is altered by pacing with ventricular electrodes instead of atrial electrodes, LVmax dp/dt decreases immediately, even in the presence of a concomitant rise in LVEDP.

(5) the inotropic agents norepinephrine and acetyl-strophanthidin cause an increase in LVmax dp/dt in the presence of a fall in LVEDP.

LVmax dp/dt can therefore be utilized to serve as an index of extrinsically induced changes in myocardial contractility. Interpretation of changes in LVmax dp/dt can be done only after taking into consideration alterations in heart rate, aortic pressure and LVEDP. Reeves and Hefner also showed, using direct measurements of myocardial tension by a Walton-Brodie strain gauge, that in response to inotropic interventions max dT/dt, total tension and the Tension Time Index, obtained by multiplying tension by rate, were increased.(72) In their experiments max dT/dt consistently revealed greater changes than the other measurements.

Rate:

The influence of heart rate on LVmax dp/dt has been critically evaluated by Mitchell, Wallace and Skinner(73) who came to the same conclusions as the previously mentioned authors. Their work was however performed in the right heart by-pass prepara-

tion. This same aspect has since been investigated in the conscious dog by Noble et al[74] and the same generalizations were found to be applicable in that LVmax dp/dt increases linearly with heart rate in the presence of controlled afterload and initial fibre length.

Afterload:

The influence of the afterload, i.e. aortic pressure, on the rate of development of left ventricular pressure was first realized by Wiggers.[6] He indicated the need for control of this variable in the assessment of contractile responses. Wallace et al[71] investigated the role of the afterload during conditions of constant stroke volume and heart rate. Elevation of mean aortic pressure resulted in a linear increase in LVmax dp/dt whether or not LVEDP rose. When aortic resistance was abruptly increased between two consecutive contractions, such that the first contraction after elevation was initiated from the same LVEDP, a linear relationship was once again demonstrated between LVmax dp/dt and mean pressures, between 50 and 130 mm Hg. These authors found an equally good correlation of LVmax dp/dt with aortic diastolic and aortic mean pressure.

LVEDP:

This was shown to influence LVmax dp/dt in virtually predictable fashion according to the Frank-Starling principle. The definitive studies are commented on by all the aforementioned groups of authors.[70,71,72,73]

Reeves et al[70] demonstrated that the infusion of methoxamine, a sympathomimetic agent with virtually pure alpha-adrenergic activity and the effects of which on the heart are

produced through alterations in the peripheral vasculature only, resulted in an increase in LVmax dp/dt as well as an increase in systolic pressure. From their published data it is evident though that a relatively large increase in LVEDP is required to effect an increase in LVmax dp/dt.

(c) LVmax dp/dt as an Index of Myocardial Contractility:

The behaviour of LVmax dp/dt with haemodynamic alterations and inotropic interventions have been discussed and the similarity between its changes and those of the force-velocity relation and in particular Vmax, is evident. The fidelity with which LVmax dp/dt reflects alterations in the force-velocity relation and hence the basic contractile state of the myocardium, has been the subject of several recent investigations.
The assumptions involved in extrapolating force generation to tension development and pressure measurement have been outlined by Badeer.(75)

The peak rate of tension generation in the myocardial fibres, as reflected by pressure generation, occurs during the midportion of the isometric phase of the ventricular pressure curve. This is indicated in Fig.14 obtained from a high sensitivity photographic recording in a dog during an inotropic stimulus, an infusion of isoproterenol. The oscilloscopic beam here had a light source with 3600 oscillations per second. During the peak phase of isometric tension development, the recorded line is separated into its component dots on this 100 mm/sec recording. It is apparent that the greatest distance between the dots appears just prior to aortic valve opening. The highest output of a differentiator circuit, appearing as an upward deflection, occurs exactly above this section of the

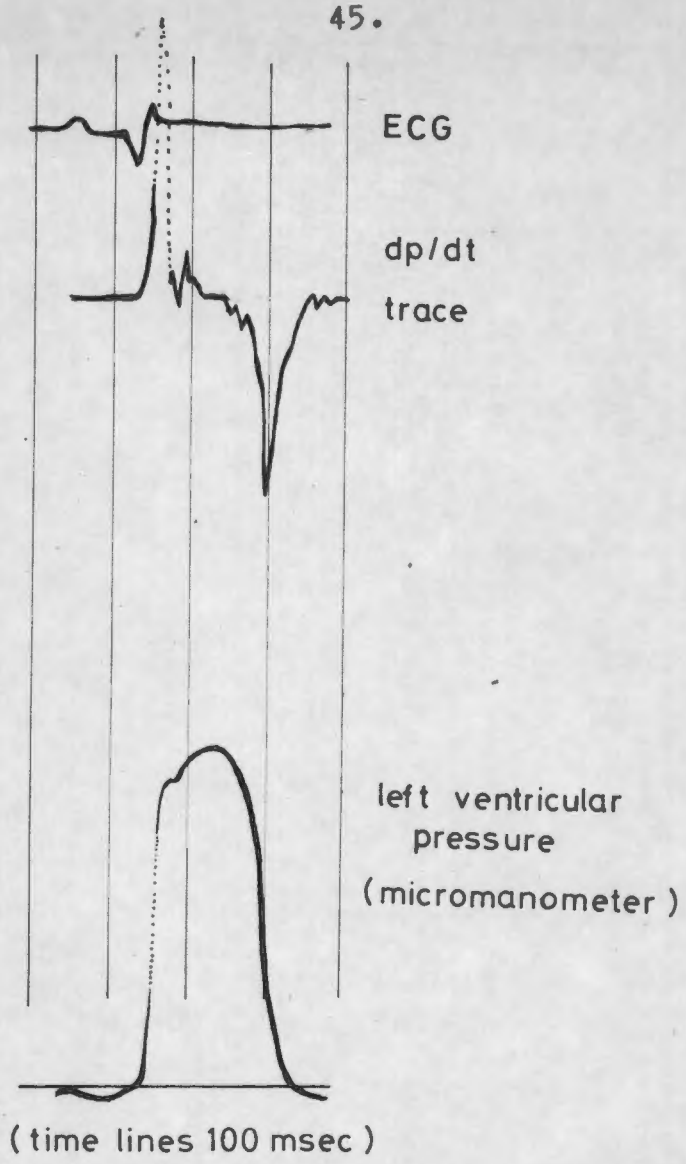


FIG. 14

curve. The electrocardiogram is also shown.

The relation of peak LV dp/dt to contractile element events was investigated by Ross et al in their study of isovolumic beats in the dog heart.[59] These authors succeeded in relating LVmax dp/dt to the time course of the velocity of contractile element action as shown in the composite Fig.15 (taken from Ross et al[59]).

This shows the time course of events occurring during three single contractions, one being isovolumic (open squares), one against a relatively high afterload (control, open triangles), and one against a lower afterload (unloaded, closed circles). The relation of peak dp/dt to aortic ejection and left

ventricular pressure generation is clearly demonstrated.

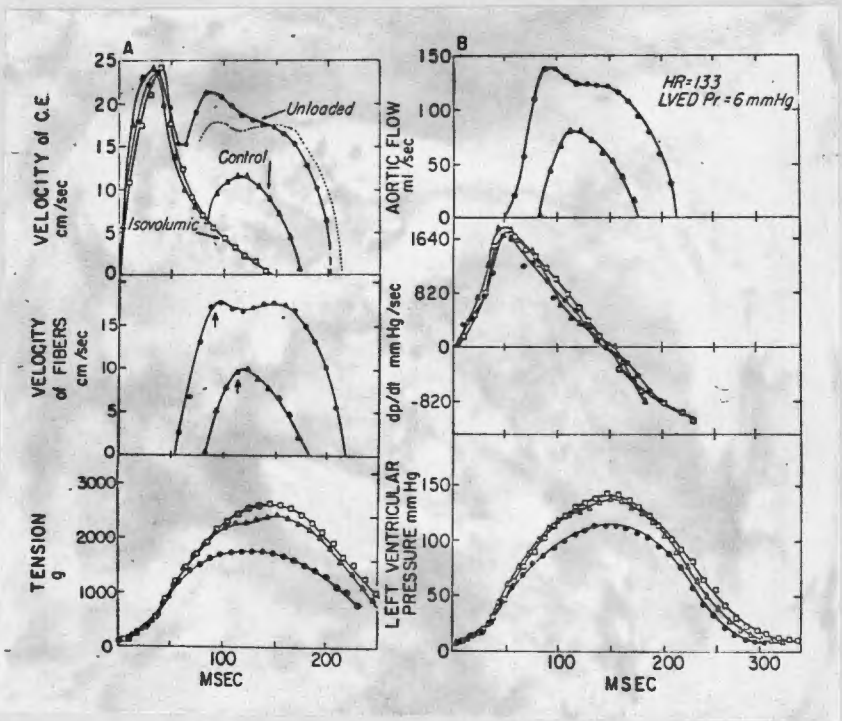


FIG. 15

Deserving of comment is the fact that in the isovolumic beat in which ejection was prevented, the peak dp/dt coincided exactly with that in the beats in which ejection occurred.

In a recently reported comparative study, Covell et al[76] critically evaluated the value of the force-velocity curve obtained in single isovolumic beats, the peak rate of rise of left ventricular pressure (LVmax dp/dt) and the ventricular function curves relating LVEDP and stroke work and power, as indices of contractility in the canine right heart by-pass preparation. Stimuli of alterations in heart rate, norepinephrine infusions and hypothermia were employed to change the contractile state. These authors demonstrated that the force-velocity curve measured in isovolumic beats, is the most sensitive index of myocardial contractility, but significantly demonstrated that LVmax dp/dt was not greatly inferior. This measurement revealed smaller, but distinctly comparable

changes during all their alterations in inotropic state and was greatly superior to the work or power v.s. LVEDP curves. During several subtle alterations in contractile state, the former measurements revealed changes whereas the latter ones remained unchanged.

Tolman and Young[77] using the heart-lung preparation also measured simultaneous changes in the slope of left ventricular pressure tracings and alterations in stroke work v.s. LVEDP and came to the same conclusions. A similar study was also recently reported in the conscious sedated dog by Taylor et al[60] who measured left ventricular pressure and its first derivative through a short polyethylene cannula inserted directly through the chest wall. Isovolumic beats were obtained by a power-driven balloon situated just above the aortic valve on a catheter. Their conclusions correlated well with those of Covell[76] and Tolman and Young.[77]

These various groups of investigators all demonstrated that although not as accurate as actual force-velocity curves, LVmax dp/dt closely approximates the more sophisticated measurements as an index of myocardial contractile state and is more sensitive than the curves relating stroke output, work or power to LVEDP. These conclusions serve to confirm the views previously expressed by Rushmer.[78]

The use of LVmax dp/dt as a measure of myocardial contractility has been reviewed by Siegel et al.[79] These authors have proceeded to show how, by the use of time-tension relationships during the isometric phase of left ventricular contraction, LVmax dp/dt, as a measure of contractility, can be amplified.[80] Out of their observations emerged the fact

that a fundamental difference can be identified between the way in which myocardial isometric force is developed by the Frank-Starling mechanism and by a primary change of contractility in the contractile element. Using the right heart by-pass preparation and isolated papillary muscles, these authors demonstrated that at a constant rate of stimulation, increments in initial length produced no alteration in the duration of the rising phase of isometric tension (taken from onset of tension rise to peak tension). Both the maximum integrated systolic isometric tension (IIT), obtained by planimetrically integrating the area beneath the rising phase of the isometric contraction, as well as the maximum rate of development of the tension, (or pressure) dp/dt increased with increases in end-diastolic volume and fibre length. The ratio $\frac{dp/dt}{IIT}$ remained constant therefore over the entire range of fibre lengths studied. In the presence of an increase in myocardial contractility, the ratio $\frac{dp/dt}{IIT}$ increased to a new constant which was independent of fibre length. Unlike increases in initial length, in which both dp/dt and IIT increased in parallel, keeping a constant ratio, with an inotropic intervention, dp/dt increased much more than IIT so that the ratio $\frac{dp/dt}{IIT}$ increased. An increase in rate alone also resulted in an increase in the ratio $\frac{dp/dt}{IIT}$. These authors suggest that these observations are consonant with force-velocity data in that increments in fibre length, which increase Po without increasing Vmax, also do not alter the ratio $\frac{dp/dt}{IIT}$. They further propose that the net effect of examining the ratio $\frac{dp/dt}{IIT}$ is to eliminate the effect of changes in Po produced by fibre length alone so that the index is altered only by changes in Vmax relative to Po i.e. by true primary alterations in

contractility.

Clinical application of the measurement of dp/dt has been reported by Gleason and Braunwald.[81] These authors examined the first derivative of the ventricular pressure pulse in human subjects by monitoring pressure with a needle introduced directly through the chest wall. A limited number of observations were also obtained by an intracardiac micromanometer situated at the tip of a cardiac catheter. These authors demonstrated an increase in LVmax dp/dt in the human heart with infusion of isoproterenol, exercise and after ventricular premature contractions. Unlike the effects of acute elevations of ventricular end-diastolic volume, haemodynamic abnormalities that result in a chronic augmentation of ventricular stroke volume, did not result in abnormal values for LVmax dp/dt. It was found, however, to correlate well with the peak systolic pressure chronically developed by the ventricle. It was postulated that the high LVmax dp/dt observed in ventricles that developed elevated pressures reflect the increased muscle mass that is present. This is consonant with the views of Sandler et al[82] who presented evidence that the thickness of the ventricular wall increases in proportion to the systolic pressure chronically developed by the ventricle. Patients who had experienced congestive heart failure or who had roentgenographic evidence of left ventricular enlargement and elevation of the ventricular end-diastolic pressure tended to have lower values for LVmax dp/dt than might have been expected according to their systolic pressure and heart rates. This may be explained according to the Law of La Place as outlined by Rushmer[83] and Gorlin.[84] It is apparent that any given rate of development of tension by

the myocardium in a dilated heart, would result in a slower rate of pressure rise and, hence, in a low LVmax dp/dt. Buckley and Zeig[85] have also demonstrated in the isolated heart that acute left ventricular failure is accompanied by a decrease in the rate of ventricular pressure rise.

(d) TECHNICAL ASPECTS OF THE MEASUREMENT OF dp/dt IN THE INTACT HEART.

Differentiation:

The maximum rate of rise of pressure in the left ventricle can be obtained either by manual estimation of the steepest slope of the left ventricular pressure curve on high-sensitivity, high-speed tracings or by electronic differentiation of the output of an electronic pressure transducer. This can be accurately and automatically achieved in continuous fashion by the R-C circuit. The output of such a circuit is proportional to the first time derivative of the sine wave of the left ventricular pressure course. The components of the R-C circuit are indicated in Fig.16.[86]

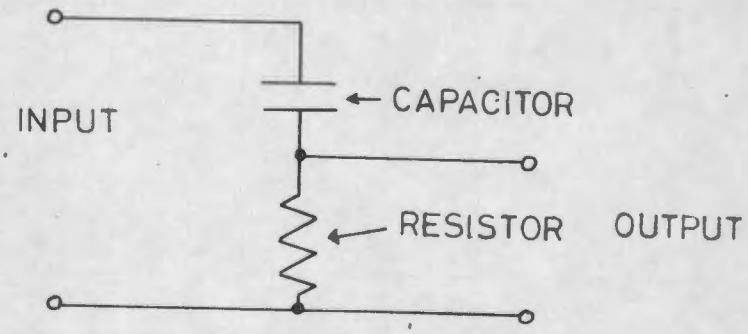


FIG. 16

If a current is passed through such a circuit, the total voltage drop will occur across the resistor. A certain phase lag will be imparted by such a circuit (with reference to the original current). Negative charges will accumulate on one

side of the capacitor. As the charge accumulates, the voltage across the capacitor increases and the current in the circuit decreases. Since the sum of the voltage across the resistor and the capacitor must equal the original applied voltage, when the voltage across the capacitor increases, the voltage across the resistor must decrease.. This decay will occur at a rate proportional to the time-constant of such a circuit. A graphic description of the series of events, using square-wave changes in voltage are shown in Fig.17. The effect of a reduction in the time constant is also indicated.

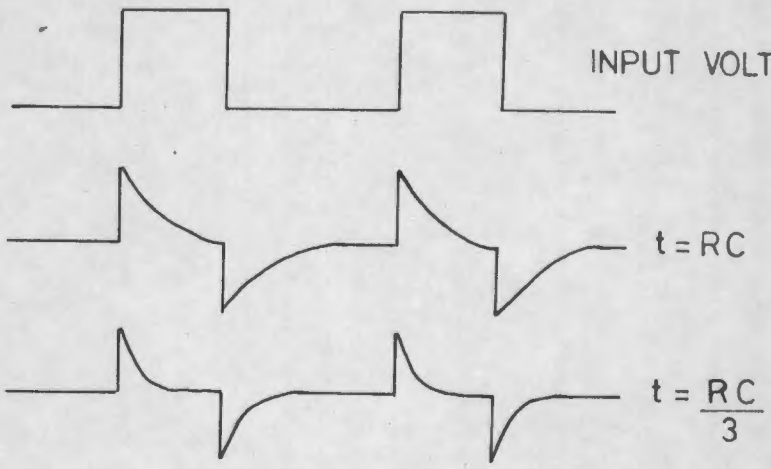


FIG. 17

By means of such a circuit, the first derivative with respect to time of the pressure pulse can be determined at any instant in the cardiac cycle. The time-constant of such circuits are usually of the order of 0.1 to 0.5 msec, providing differentiation with output frequency linear to input frequency in the range 0-75 cycles per sec.

Recording systems:

The limitations of recording systems used in the measurement of cardiovascular phenomena have been outlined by Wood.(87)

In a detailed analysis of the requirements of recording systems Fry has set the following criteria for such a system:[88]

(i) it must have static accuracy i.e. it must have stability and be free of drift from a constant baseline.

(ii) it must have dynamic accuracy i.e. it must simulate the dynamic events with fidelity. This implies that it must be free from noise artifacts, endogenous or exogenous.

(iii) it must have low physiological reactance i.e. it must produce minimal interference with the events due to its physical presence.

Since such systems are comprised of (a) a transducer (b) an amplifier and (c) a recorder, the fidelity of such a system, and particularly its amplitude response (amplitude rati at any given frequency) will be equal to the product of the amplitude response of each of the components.

Modern equipment consist of transducers with sufficient dynamic accuracy to have a linear response at frequencies well beyond the limits of any cardiovascular phenomena. The amplifier and recorder systems, particularly if oscilloscopic syster with photographic recording is used, can for all practical purposes be considered as not contributing to any distortion o the signal, apart from the possible introduction of noise signals. These are of particular importance if high amplification ratios are used but may be partially attenuated by the introduction of electronic filters into the circuit.

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The limiting factor of importance in all these systems therefore is confined to the method of connection of the trans

ducer to the system being monitored e.g. a catheter system. Micromanometer systems have recently been devised whereby the transducer, built into the tip of a catheter, can be introduced into the required situation where pressure is to be sensed. These micromanometers, apart from being expensive, have certain limitations which preclude their use under most circumstances, notably the inability to be calibrated according to a zero standard while in situ.

Limitations of Catheter-manometer Systems:

Catheters have great limitations that contribute to the inaccurate recording of physiological events. These have been discussed by Fry[88] and by Franke.[89] The use of such a probe as a transmission system almost always degrades the dynamic response of the transducer system since in general the physiological information will be distorted in transit through the probe and noise, generated from extraneous sources along the probe, will appear at the transducer element indistinguishably from the physiological information. Characteristically the pressure-monitoring system has a fluid-filled column between the blood vessel or ventricular cavity and the transducer membrane. The catheter tube usually has stiff walls and is filled with physiological saline solution. For faithful recording of pressure as a function of time, the deflection of the membrane should be directly proportional to the pressure at the catheter tip. The membrane needs to be deflected by the pressure and hence the fluid column in the catheter must be physically displaced by the pressure pulse. The inertia and viscosity of the fluid mass in the catheter will therefore enter importantly into the dynamic response of the system. The presence of air, even in microscopic bubbles, can alter the physical charac-

teristics of such a column to a profound extent. Outward displacement of the walls of the catheter will serve to attenuate the pressure wave.

Pressure recording errors in such a system arise from both the intrinsic transmission properties of the system as well as from "noise" related to extrinsic motions imparted to the system by direct contact of its walls with moving systems.

The intrinsic transmission properties may impose four groups of errors on the physiological event:-

(1) Multiple reflections of the frequency components:- frequency components approaching the various "resonant frequencies" of the system may be greatly amplified because of summations of reflected waves with incoming waves if they are in phase.

(2) Unequal attentuation of the different frequency components during transmission.

(3) Different transmission times for each frequency component.

(4) Wave generation from extrinsic motions.

The need for assessment of the fidelity of such a system is therefore evident. The dynamic accuracy of such a system may be determined from its frequency response curve. This will be outlined in detail in the Methods section.

Since the first derivative of ventricular pressure is a function of frequency, rather than amplitude, the degree of damping in the catheter system is of utmost importance. Any significant damping will retard the upstroke of the pressure curve. The importance of using systems with low damping ratios is thus desirable, but such systems are often non-linear for

amplitude even at low frequencies. The contribution of non-linearity for amplitude to the recording of the derivatives of pressure is not known. Knopp et al[90] comparing a short tube directly inserted into the left ventricle with a conventional catheter system, found that the catheter-manometer system tended to underestimate rather than overestimate LVmax dp/dt. Their catheter-manometer system, however, was operated with the output passing through a 15 cps filter and the contribution of this enters another variable.

The frequency range of dynamic events in the cardiovascular system has been variously estimated to lie between 0-20 cps by Wiggers[91], 0-33 cps by Klip[92] and even 0-100 cps by McDonald.[93] The latter two estimations are, however, based on theoretical considerations. McDonald in an extensive series of Fourier analyses of cardiovascular wave forms, suggests that for practical purposes, a range of 0-20 cps is adequate.[93] Knopp et al[90] used a system linear to 90 cps and conducted Fourier analyses on their recordings. They found that 95% of the energy components of significance occurred at 15 cps or less, even at high heart rates. Their estimations included direct measurements of LVmax dp/dt.

3. THE AIMS OF THESE INVESTIGATIONS.

The factors known to influence myocardial contractility have been discussed and a method suitable for the assessment of the contractile state of the myocardium in the intact animal has been outlined.

It has been known for a long time that thyroid diseases are associated with profound cardiac manifestations. The hyperthyroid state is associated with a hyperkinetic circulatory derangement whereas the opposite situation holds in hypothyroid conditions. The incidence of cardiac disease in both situations is much greater than is commonly appreciated.(94)

Two mechanisms for the haemodynamic manifestations of thyro-cardiac diseases have been invoked. Firstly the hormone may have a direct effect on the myocardium and secondly it may act by sensitization of the heart muscle to the effects of the sympathetic nervous system and the catecholamines. An extensive literature, recently reviewed by Harrison(95) has arisen attempting to prove either or both of these mechanisms.

The purpose of this thesis and the experiments described is to assess the role of thyroid hormone as a possible determinant of myocardial contractility in the intact animal. The animal model used was the intact conscious dog in an effort to eliminate the variable effects of anaesthesia. Myocardial contractility was assessed primarily by the estimation of LVmax dp/dt, in an attempt to gain insight into the force-velocity relations of the myocardium under the influence of thyroid hormone. In addition the more conventional expression of contractility in terms of output, rate of output, work and power in relation to

LVEDP was obtained simultaneously. Contractility is assessed according to the conditions outlined by Blinks[96] and by Blinks and Koch-Weser.[97]

In this fashion it is hoped to establish whether effects of the hormone are in a primary fashion affecting the fundamental properties of the contractile machine or whether the changes in performance arise solely secondary to effects removed from the heart.

Using the dog with experimentally induced hypo- and hyperthyrodism, the alterations in myocardial contractility attributable to changes in thyroid status are defined. An attempt is then made to characterize the increased contractility encountered in the hyperthyroid state in terms of fractions associated with the increase in heart rate and that mediated directly by the hormone. By means of the beta-adrenergic blocking agent, propranolol, the increase in contractility in the hyperthyroid state is further analysed into a fraction due to increased stimulation of beta-receptors by endogenous catecholamines and the fraction induced by a direct action of the hormone.

The influence of alterations of thyroid state on the contractile responses to beta-adrenergic stimulation was investigated by infusions of isoproterenol in normal, hypothyroid and hyperthyroid dogs. Thyroxine (T4) was used to induce the hyperthyroid state; experiments were also performed with triiodothyronine (T3) to demonstrate the rapidity with which thyroid effects on myocardial contractility could occur.

4. PROTOCOLS, MATERIALS AND METHODS.

The level of myocardial contractility was measured in normal adult dogs, in dogs rendered hypothyroid by the administration of I^{131} and in dogs treated with thyroid hormone until hyperthyroid. All animals were kept under the same conditions at the facilities of the New England Primate Research Centre, Southboro, Massachusetts.

Normal dogs:

These were both males and females randomly selected from a pool of animals reared under the same conditions. The animals had to appear normal in all respects and had to be free from infected wounds. Autopsy confirmation of normality at the end of experiments was obtained in all animals. All dogs with heartworm at autopsy were excluded from the series.

Hypothyroid dogs:

These dogs were from the same pool, but had been treated with I^{131} from six weeks to twelve months prior to study. These animals had commenced to gain weight and had elevated serum cholesterol levels, but did not show the external manifestations of gross myxoedema.

Myxoedematous dogs:

These animals had been given I^{131} two years prior to study. Myxoedema as evidenced by marked weight gain, a dry scaly skin with lard-like subcutaneous tissues and failure of hair growth after shaving was present in all animals. Serum cholesterol levels were grossly elevated.

Hyperthyroid dogs:

These animals were randomly selected from the pool of dogs and

were studied in the normal state. Following the baseline study, they were given l-thyroxine by subcutaneous injection daily for periods ranging from one to four weeks.

(a) Induction of Hypothyrodism:

Animals were given I^{131} by intravenous injection. A single dose of 25 millicuries was given after obtaining baseline weight and blood samples for estimation of total serum cholesterol. A repeat dose was given three weeks later. This procedure had previously been found to render dogs hypothyroid without rapidly causing death.(98) Haemodynamic studies in these animals were performed as early as six weeks after I^{131} and as late as 2½ years after administration. The progress of the hypothyroid state was monitored by estimations of body weight and total serum cholesterol. Protein-bound-iodine estimations were initially obtained, but were discarded since all animals had radiographic procedures involving iodine-containing contrast media which rendered future estimations invalid.

Untreated dogs maintained under the same conditions retained normal levels of serum cholesterol without any weight gain in a period of observation of one year.

In order to exclude possible effects of secondary complications of hypothyroidism e.g. pericardial effusions or coronary atherosclerosis on the haemodynamic profile of these animals, coronary arteriography was performed in all animals at the conclusion of each study. After completion of the series of experiments, each animal was examined at autopsy. The heart was removed, weighed and the coronary arteries were injected with a radio-opaque injection mass of lead acetate in agar, according to the technique of Schlesinger.(99)

Roentgenograms were obtained and examined for any lesions of the coronary arteries. Pericardial effusions were not found in any of the animals. Heartworm was found in two of the dogs and these were excluded from the series.

(b) Induction of Hyperthyroid State:

Eight dogs were studied in the normal state. Treatment was then commenced with l-thyroxine sodium (l-T4). The powder was dissolved in 0.1 N Sodium hydroxide solution and diluted to the appropriate concentration. Each dog was given 0.1 mg/kg daily by subcutaneous injection. Each animal was studied after 7 days of treatment and again at three or four weeks. Since no differences were found between the studies at three and four weeks they are treated as a single group. In this fashion each dog served as its own control. A further control was obtained in one dog studied in the baseline state, after one week of thyroxine administration, after a further two weeks of the solvent for thyroxine alone and finally after a further one week of thyroxine again.

Conditions of Study:

All studies were conducted after overnight fasting. Morphine sulphate 1.5 mg/kg was given subcutaneously one hour before the commencement of the experiment. The resulting sedation was light enough to permit the animals to walk from their cages to the catheterization laboratory, but sufficient to enable catheterization to be performed without any need for restraints or muzzling.

Percutaneous catheterization of both ventricles and the aorta was performed by the Seldinger technique.(100) Two percent procaine was used as local anaesthetic.

Haemodynamic observations were obtained on two occasions fifteen minutes apart to define the baseline before any further procedures were initiated.

2. Relative Contributions of Heart Rate and Associated Autoregulatory Component and the Direct Effect of the Hormone.

In the dogs submitted to thyroxine treatment the influence of an increase in heart rate on LVmax dp/dt was assessed during the control study and after induction of the hyperthyroid state. The rate increase was achieved by electrical stimulation of the right atrium. The importance of maintaining the normal pathways of conduction in the assessment of myocardial contractility has been emphasised.(22) Rate - LVmax dp/dt curves were obtained in all dogs during the control and hyperthyroid states and the curves analysed for significant differences in elevation and slope. Information was also obtained on whether the autoregulatory mechanism itself was altered by thyroid treatment. In all studies, the pacing was performed immediately subsequent to obtaining the baseline haemodynamic observations i.e. before any drugs were administered. Complete capture of the sinus mechanism had to be obtained with a normal PR interval on the electrocardiogram before the tracings were accepted for analysis.

3. Influence of Thyroid Status on Myocardial Responses to Beta-adrenergic Stimulation.

Subsequent to obtaining baseline haemodynamic observations in all animals, infusions of the beta-adrenergic stimulant, isoproterenol were administered to all animals. This agent was chosen because of its relatively pure beta effect in comparison to both norepinephrine and epinephrine, both of which were

found to have significant alpha effects with resulting bradycardia in the intact animal. Better dose-response curves could therefore be obtained both for contractility and heart rate with isoproterenol compared to norepinephrine and epinephrine. The dose levels used ranged from 0.01 to 0.20 µg/kg/min in all animals. This rather narrow range was adhered to because of its relative freedom from both ventricular extrasystoles and restlessness in these conscious animals. Since displacement of the curve as a whole rather than extension of its extremities was involved it was found to be satisfactory. All infusions were standardized by being of twelve minutes duration with observations obtained at that time in all instances. A constant rate of infusion was maintained by means of a Braun pump.

4. Relative Contributions of Sensitization to Endogenous Catecholamines and Direct Effect of the Hormone on the Myocardium.

This was investigated in the group of dogs rendered hyperthyroid by complete pharmacological blockade of the beta-receptors with propranolol. This was done during the control euthyroid study and again after hyperthyroidism had been induced. The degree of beta-blockade obtained with 0.4 mg/kg propranolol was assessed by challenge with the highest dose of isoproterenol used in the stimulation experiments, 0.2 µg/kg.

5. Effect of l-triiodothyronine on Resting Haemodynamics and Responses to Isoproterenol.

16 normal and 6 hypothyroid animals were used to examine the influence of l-triiodothyronine (l-T3) by infusion and in another 4 dogs by single injections. Baseline observations were obtained in all animals. After this, infusions of isoproterenol were administered as usual and observations obtained.

Following a rest period of one hour during which the baseline state was again obtained, an infusion of l-triidothyronine was commenced and observations repeated after 15 minutes. The infusions of isoproterenol were then repeated in the same doses for the same periods. The experiments were controlled by infusions of the 0.1 N Sodium hydroxide used as solvent for the l-T3, diluted to the same concentrations with normal saline as when the l-T3 was added.

The experiments were repeated, in order to establish specificity for l-T3, with d-Triidothyronine in exactly the same fashion in a further four dogs.

EQUIPMENT:

Catheterization:

The catheters used were the same in all animals. Where possible the same catheter was used repeatedly in the same dog undergoing serial studies. The catheters were inserted over a P.E.160 "Kifa" guide wire (United States Catheter and Instrument Co., Glens Falls N.Y.) inserted through a 16 gauge Seldinger needle after withdrawal of the stilette. Size 6F, "Rothene II" polyvinyl catheters (Electro-catheter Corporation, Rahway, N.J.), 80 cm long with four side apertures within 1 cm from the tip were used for all experiments. The catheters were moulded into a "pig-tail" curve for left ventricular cannulation and with a gentle "C" curve for right ventricular cannulation. The two shapes are indicated in Fig.18.

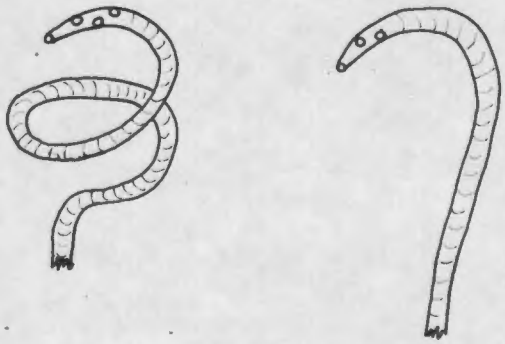


FIG. 18 L.V. R.V.

The catheter used for cannulation of the aorta was left straight. The femoral arteries were punctured after making a puncture in the skin with a pointed knife-blade, large enough to insert the needle. The catheters were inserted and positioned in the ventricles and aorta by retrograde advance under direct fluoroscopic monitoring with display on a closed-circuit television module. The aortic catheter was placed with its tip in the arch of the aorta. The catheters were connected directly through a three-way stopcock to the transducers and were

repeatedly flushed with heparinized physiological saline solution. Each dog was given 20 mg heparin at the completion of catheterization and thereafter 10 mg every hour by intravenous injection.

Pacing Instruments:

Used was a bipolar pacing catheter (United States Catheter and Instrument Co., Glens Falls, N.Y.) inserted by cutdown under local anaesthesia on either a brachial or an external jugular vein. The catheter was advanced under direct vision on the fluoroscopic screen into the right atrium and the pacing unit attached. The impulse generator used was a specially modified Electrodyne TR-6 external pacemaker, capable of delivering impulses at a rate of 0-200 per minutes (courtesy of Electrodyne Company, Westwood, Massachusetts). The minimal rate sufficient to cause repetitive stimulation was obtained and observations recorded after 5 minutes. The rate was then increased stepwise by 10 beats per minute and at each new rate haemodynamic observations obtained after 5 minutes. Tracings were discarded when A-V block developed.

Recorders and Transducers:

The electrocardiogram, left ventricular pressure at high and low sensitivity, aortic pressure, the first derivative of left ventricular pressure and a zero baseline were displayed continuously on the oscilloscopic screen of an Electronics for Medicine D.R.8 recorder, (Electronics for Medicine, White Plains, N.Y.)

Recordings were obtained at high speed (100 mm/sec) for analysis of events within the cardiac cycle and at slow speed (10 mm/sec) for accurate estimation of heart rate. The time

markings were set at 0.1 sec intervals for the high speed tracings and at 1 sec intervals for the slow speed. At least ten cycles were included in each high speed trace and the slow trace was obtained over a 30 second period, during the performance of a cardiac output estimation, in order to obtain an accurate estimation of stroke volume.

All recordings were obtained photographically and fixed for high resolution in order to obtain accurate records for estimation of ejection times.

Pressure measurements were obtained with P23dB pressure transducers, (Statham Instruments, Hato Rey, Puerto Rico), placed level with the midchest point. These transducers were calibrated against a mercury column at the start of each day.

Amplification was through SGM-2 channels of the EFM DR8 recorder. The low sensitivity pressure trace was recorded directly through the channel whereas the output was amplified by a ratio of approximately 10 through a slave channel to obtain a high sensitivity tracing for analysis of changes in left ventricular end-diastolic pressure. The low sensitivity trace gave a deflection of 35-40 mm deflection/ 100 mm Hg.

Pressure in the aorta was measured simultaneously with a second channel calibrated to equal the displacement of the channel used for left ventricular pressure measurement.

Right ventricular pressures, when recorded, were obtained through a channel with its sensitivity augmented by a factor of 4 in comparison with the left ventricular measurements.

LVmax dp/dt was obtained continuously by differentiation of the left ventricular pressure signal by the built-in R-C

saline. Blood was withdrawn from the aortic catheter at the rate of 50 ml/min by means of a constant-rate Gilford withdrawal system (Gilford Instrument Laboratories Inc., Oberlin, Ohio). The blood was passed through a Gilford cuvette densitometer and the output of this recorded with a Texas Instruments "Recti-Riter" direct-writing ink recorder. (Texas Instruments Inc., Houston, Texas). The speed of recording was 1 cm per sec enabling manual integration of the dye-dilution curves to be performed at 0.5 sec intervals. A concentration curve for the dye was obtained at the conclusion of each experiment with the same dye used in the experiments and the blood of the particular animal. Four points were obtained in all these concentration curves. Curves were replotted on a semi-logarithmic scale with the initial portion of the down-slope extrapolated to meet the X axis in order to exclude the effects of recirculation and arrive at an accurate estimate of the transit time. The cardiac output was obtained from the formula:

$$Q = \frac{I}{\int Ct.dt} \times 60$$

where Q = output in ml/min.

I = amount of indicator injected in mg.

$\int Ct.dt$ = area under dye curve. (mg/ml.sec)[102]

Micromanometer:

This was used in six experiments to test the adequacy of the frequency-responses of the catheter-manometer system. The model SF 1 catheter-tip transducer (Statham Instruments, Hato Rey, Puerto Rico) was inserted into the left ventricle by

retrograde advance after insertion through a cut-down on a brachial artery under local anaesthesia. The pressure recordings were obtained simultaneously with those obtained through the catheter-manometer system. The outputs of both systems were differentiated and recorded simultaneously.

Calibration of the micromanometer was performed in situ against the catheter-manometer system and its derivative output was calibrated manually on 200 mm/sec recordings done at high sensitivity.

CALCULATIONS:

(1) Heart rate:

This was obtained from 30 sec slow traces at 10 mm/sec by manual counting. When artificial pacing was employed, the interval between stimuli was measured on 100 mm/sec tracings and the rate calculated as

$$\underset{(\text{per min})}{\text{Rate}} = 60 \times \frac{1}{\underset{(\text{Sec})}{\text{Stimulus interval}}}$$

(2) Pressures:

These were read off directly against calibration scales applied electronically at the beginning and end of each experiment. LVEDP was read against its own calibration scale with the junction of the "a" wave and the commencement of the rapid upstroke of LV pressure, taken as the reading. The first derivative of pressure was obtained by manual measurement of the displacement of the beam and the reading obtained in mm Hg/sec as outlined above. Mean aortic pressure (MAP) was calculated as follows:

$$\text{MAP} = \frac{\text{Systolic pressure} - \text{Diastolic pressure}}{3} + \underset{(\text{in mm Hg})}{\text{Diastolic pressure}}$$

(3) Ejection Time:

This was obtained by direct measurement from the 100 mm/sec tracings of aortic pressure. The interval between the commencement of pressure increase in the aorta and the nadir of the diastolic notch was measured and calibrated according to the 0.1 sec time markings on the recording. The first few cycles of each period were excluded to avoid errors due to the initial acceleration of the paper moving at such great speed.

(4) Stroke Volume and Stroke Volume Index:

This was obtained by dividing the cardiac output (in ml/min) by the heart rate per minute.

$$S.V. = \frac{C.O.}{H.R.} \text{ in ml.}$$

The stroke volume index was calculated in those instances where comparisons were made between various dogs.

$$S.V.I. = \frac{\text{Cardiac index}}{\text{Heart rate}} \text{ in } ml/m^2$$

The cardiac index was obtained from the cardiac output according to the formula:

$$\text{Cardiac index} = \frac{\text{Cardiac output}}{\text{Surface area}} \ ml/m^2/min$$

Where surface area = 0.112 x Wt. in $kg^{2/3}$ (103)

(5) Stroke Work and Stroke Work Index:

The work performed by the ventricle during each beat was calculated as the product of stroke volume and mean aortic pressure.

$$\text{Since work (W)} = \text{Force (F)} \times \text{Distance (D)}$$

$$\text{and pressure (P)} = \frac{\text{Force (F)}}{\text{Area (A)}}$$

$$\text{while volume (V)} = \text{Distance (D)} \times \text{Area (A)}$$

the equation for Work can be written

$$W = \frac{F}{A} \times D.A.$$

$$= P \times V$$

The work of the ventricle consists of the sum of two components i.e. the work performed in overcoming the pressure against which the blood is ejected and the work done in imparting velocity to the blood ejected as represented by the formula originally proposed by Frank.(23)

$$W = \int_{To}^{T_1} P.dV + \int_{To}^{T_1} d\,\frac{Mv^2}{2g}$$

where To - T_1 is the beginning and end of aortic ejection

P is the integrated mean pressure operating during this period

V is the volume of blood ejected i.e. stroke volume

M is the weight of the blood ejected

V is the mean velocity attained by the blood and

g is the gravitational constant.

The second component i.e. the kinetic component is for practical purposes about 2% of the total work(104,105) and since the use of the arithmetic mean pressure rather than the systolic pressure integrated with respect to time, reduces the accuracy of the calculation by about 10%, it was elected to disregard the kinetic component and calculate only the "pressure energy" component.

The formula was therefore -

$$SW = \frac{(MAP - LVEDP) \times SV \times 13.6}{1000}$$

where SW = stroke work in Gm meters

MAP = mean aortic pressure in mm Hg

LVEDP = left ventricular end-diastolic pressure in mm Hg

SV = stroke volume in ml

13.6 = factor to convert mm Hg to cm H_2O

The resulting answer is divided by 1000 to obtain the more useful dimensions of gm.meters.

Stroke work index (SWI) was obtained by substituting the stroke volume index (SVI) for stroke volume in the above equation. It is expressed in Gm/m

Stroke Power and Stroke Power Index:

The rate of performance of work during the period of ejection was calculated:

$$\text{Stroke Power (S.P.)} = \frac{\text{Stroke work (S.W.)}}{\text{Ejection time (E.T.)}}$$

in gm.meters/sec.

The stroke power index was obtained by substituting the stroke work index for stroke work in the above equation. It is expressed in gm/m/systolic sec.

The power of the left ventricle indicates the rate at which the left ventricle performs work as the continuous product of aortic flow and pressure. This effective power is not a measure of the total rate of energy liberation because the viscous losses within the myocardium, which are probably not negligible, are not measured. Instead the power measurement may be interpreted as a measure of the rate at which energy is transferred from the left ventricle to the peripheral vasculature.(106)

Systolic Ejection Rate:

This was calculated according to the formula:

$$\text{Systolic ejection rate (S.E.R.)} = \frac{\text{Stroke Volume}}{\text{Ejection Time}}$$

with the index, expressed as the mean systolic ejection rate (MSER), calculated using the stroke volume index.(107)

Total Peripheral Resistance and Index:

The relationship between pressure and flow for the circulation as a whole was expressed by the ratio

$$\text{Total peripheral resistance} = \frac{\text{Mean pressure in system}}{\text{Flow rate}}$$

Mean aortic pressure was used as the pressure value and converted from mm Hg to dynes/cm^2 by multiplication with 1332. The flow rate was calculated as the cardiac output expressed as ml/sec, giving the formula

$$TPR = \frac{MAP \times 1332}{Q}$$

where TPR = Total peripheral resistance in dyn.sec/cm^5

MAP = Mean arterial pressure in mm Hg

Q = Cardiac output in ml/sec

The peripheral resistance index (T.P.R.I.) was obtained by using the cardiac index expressed in ml/sec/m^2

$$T.P.R.I. = \frac{MAP \times 1332}{Cl/sec} \text{ in dyn.sec.cm}^{-5}.m^{-2}$$

Serum Total Cholesterol:

These were performed by the method of Sackett using the Lieberman-Burchardt reaction. Colorimetric determinations were performed on a Klett-Summerson photoelectric colorimeter.(108) All determinations were done by one technician (J.M.B.) who received the specimens in coded fashion without the information on the thyroid state of the particular animal.

Statistical Analysis:

The statistical approach utilized throughout was an analysis of variance. This permitted longitudinal and horizontal analysis with in-group and between-groups comparisons e.g. isoproterenol responses in normal dogs could be compared to responses in hypothyroid dogs.

This method was chosen not only for the reason stated above, but also for its particular applicability to large groups of readings. The method was used as outlined by Snedecor and Cochran.[109] The individual manipulations adapted to the requirements of the various categories are indicated in full in the Results section. The conventional abbreviations used during statistical manipulations are indicated in Table I of the Appendix.

All analyses were performed manually by the author with the use of a standard Monroe Electronic Calculator without the aid of programming functions.

FREQUENCY-RESPONSE CHARACTERISTICS OF THE CATHETER-MANOMETER SYSTEM

The need for establishing with certainty that the frequency-response characteristics of the pressure monitoring system is adequate has already been emphasized. However, since the influence of non-linearity for amplitude on the frequency-dependent phenomenon is essentially unknown, the approach to this problem was to obtain not only the frequency-response limits of the system, but also to compare the system as a whole with a system with known linearity well beyond the requirements for cardiovascular recording.

The method used was to test the dynamic response characteristics of a typical catheter used in these experiments according to the method outlined by Wood.[87] This consists of the application of a square-wave change of pressure to the system by rupturing a highly-inflated balloon with a sharply concentrated source of intense heat. The more complicated method and apparatus described by Linden[110] was thought not to be necessary since the important assessment was the direct comparison with the micromanometer system.

A rubber balloon was placed over the dome of a transducer. The side aperture was connected to an air pump and the tip of the catheter tested was introduced so that all the side apertures were exposed within the balloon. The rest of the system was set up as for standard experimental recordings. The rubber balloon was inflated to a pressure of 60 to 70 mm Hg and was ruptured using a white-hot cautery needle. The drop in pressure, which appeared instantaneous, was recorded at 200 mm/sec. A typical response is shown in Fig.19. (diagrammatically).

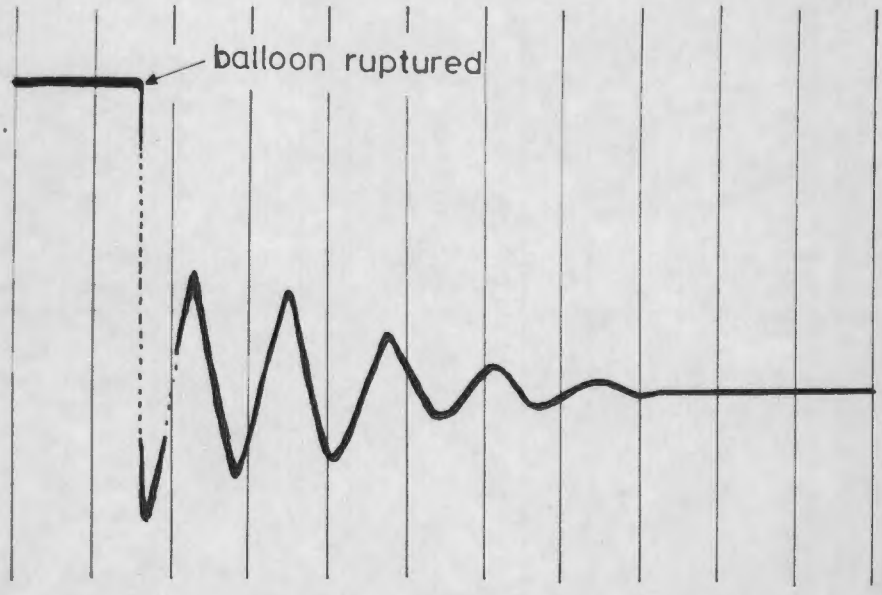


FIG. 19

Time lines 20 msec.

From such a record the required data for calculation of the frequency-response performance were obtained.

In linear systems, the response to a rectangular wave bears a relatively simple and unique relationship to the frequency-response curve of the system. If the system is sufficiently underdamped it will change rapidly to the new value about which it will oscillate at its natural frequency. The oscillations will die away at a rate determined by the amount of dampening present in the system. The frequency at which the system oscillates and the rate at which the oscillations die away are the data required for computation of the theoretical frequency-response curve of the system. The uniform portion of the curve defines the frequencies that will be accurately reproduced by the system.

One therefore obtains

(1) the dampening ratio (h) of the system. This is a measure of the amount of damping actually present compared to the critical damping (when no aftervibrations will occur).

(2) the undamped natural frequency (Wu) of the system. This would be the frequency of the free vibrations if no damping occurred e.g. if the system was frictionless.

The undamped natural frequency can be obtained in indirect fashion from the damped natural frequency. The absolute frequency (W) is related to the undamped natural frequency by a constant ratio (β).

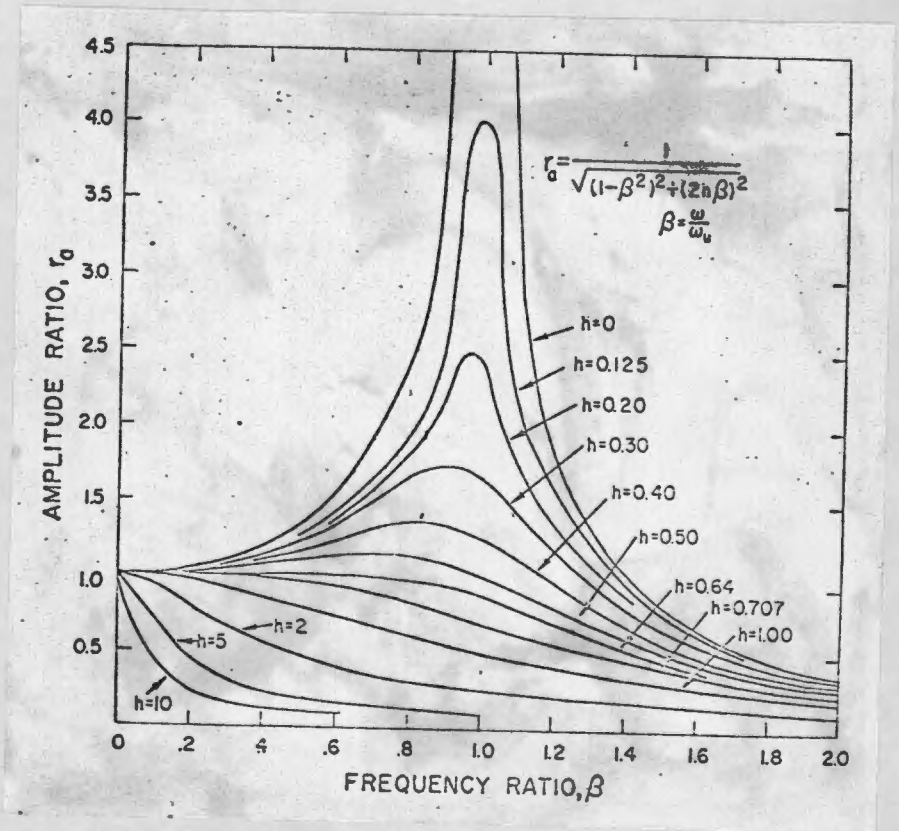
$$\beta = W / Wu$$

β and h enter into a relationship to determine the amplitude ratio (Ra) of the system.

$$Ra = \frac{1}{\sqrt{(1-\beta^2)^2 + (2\,h\,\beta)^2}}$$

The amplitude ratio must approach 1 for a system that truly measures the driving force without being affected by the frequency-response of the system.

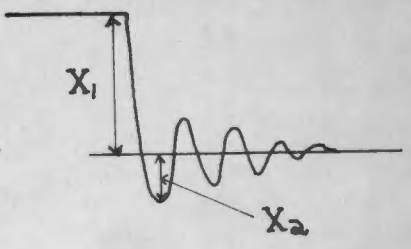
The frequency ratio/amplitude ratios are related according to constant relationships determined by the damping ratio as is illustrated in Fig. 20.(88)



In practice it is quite sufficient to determine only the frequency-amplitude ratio and not frequency-phase lag ratios of such a system.

The calculations are performed as follows:

$$h = \sqrt{\frac{\ln_e^2\left(\frac{X2}{X1}\right)}{\pi^2 + \ln_e^2\left(\frac{X2}{X1}\right)}}$$



The value for h may also be obtained from standard curves available relating the percentage overshoot to the damping ratio (Fig.21).

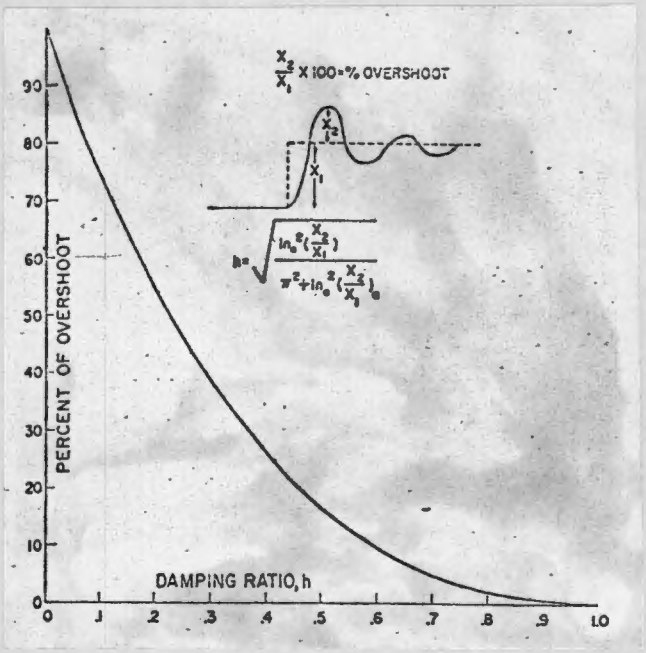


FIG.21

Once h is known the undamped natural frequency may be computed

$$Wu = \frac{Wd}{\sqrt{1 - h^2}} \quad \text{cycles/sec}$$

where Wd is the damped natural frequency, obtained by counting the frequency of the aftervibrations in cps. Once h and Wu are known, the frequency-amplitude ratio curves (Fig.20) for the specific value of h are consulted.

The frequency ratio, β, obtained from the curve may then be used to calculate the absolute frequency of the system

$$\beta = \frac{W}{Wu}$$

$$\therefore \quad W = \beta . Wu$$

This will indicate the frequency to which the amplitude response of the system may be considered uniform (usually ± 5%).

The procedure was followed for 12 separate estimations of the frequency-response characteristics. From the analysis it emerged that the "h" values were extremely low since no damping was applied to the systems in order not to interfere with the frequency-dependent estimations of LVmax dp/dt. As a result the mean value to which this system could be regarded as uniform was 12 cps. This value is not out of line with the requirements for measurements of cardiovascular phenomena indicated by Knopp et al.(90)

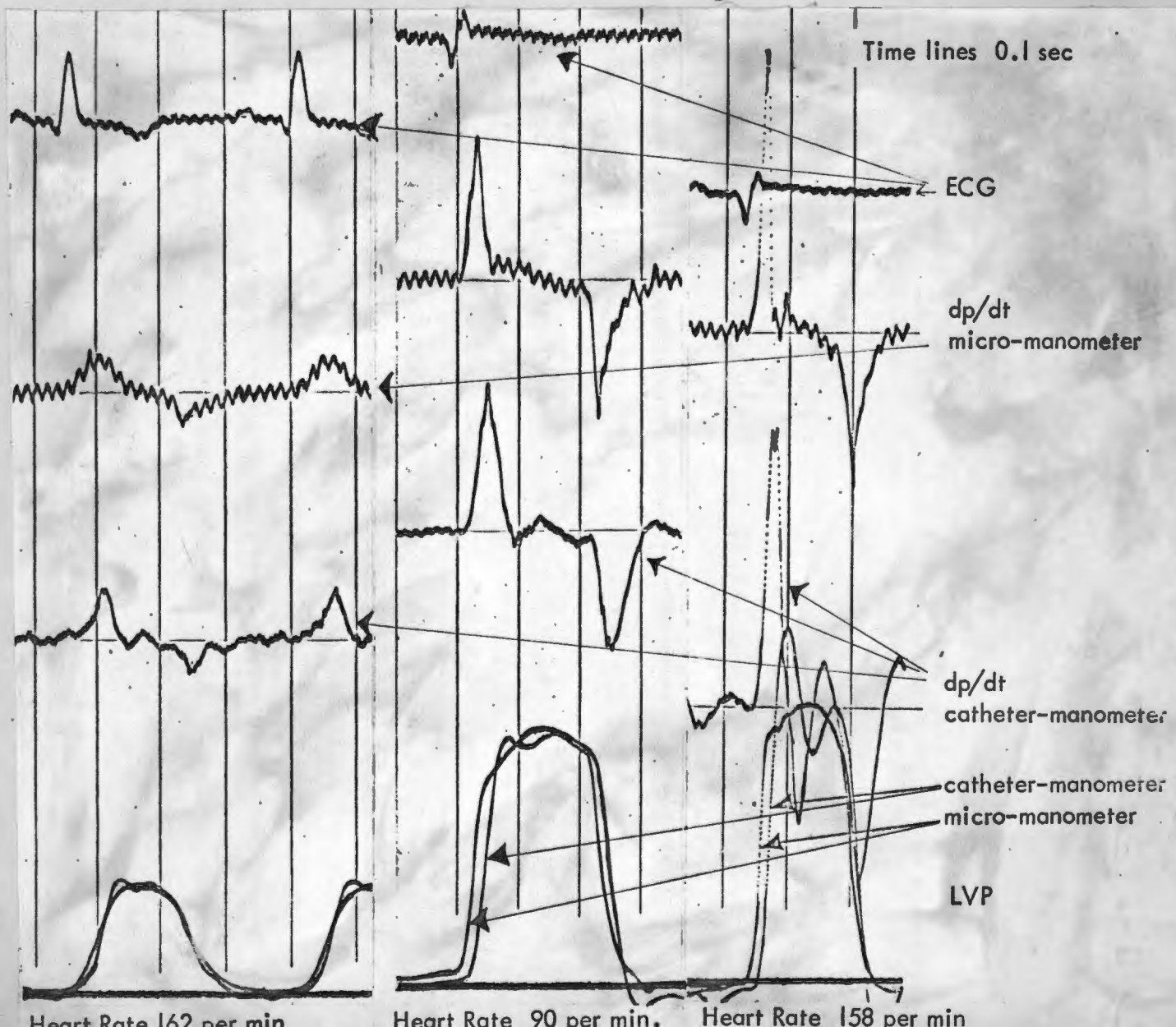
However, since the influence of non-linearity of such a system for amplitude on LVmax dp/dt could not be assessed in this fashion, it was thought to be more important to do direct comparison of the system used in these experiments with a system known to provide uniform responses to an extent well beyond ordinary requirements.

Such a system is provided by a micromanometer which can be introduced directly into the ventricle since it is built into a catheter-tip. The Statham model SF-1 transducer was therefore used. The measured linearity of this instrument has been assessed to be uniform to levels of ± 800 cps.(89)

In 6 separate experiments left ventricles were catheterized as described previously with the standard catheters used in all experiments. The amplifying and differentiating circuits remained unaltered from the usual experiments. A catheter-tip manometer was then introduced into a brachial artery by cutdown under local anaesthesia and advanced into the left ventricle in retrograde fashion and was connected to a second SGM-2 amplifying-

differentiating system. The pressures recorded by the two systems and their respective derivatives were recorded on the same record at 100 mm/sec.

In order to cover a range of pressures and rates, the dogs were subjected to isoproterenol stimulation, atropinization, pentobarbital anaesthesia and methoxamine infusions. The tracings were analysed for pressure and LVmax dp/dt in each system. Pressure readings were not analysed after seeing that the tracings coincided at peak pressures under all circumstances, confirming the adequacy of the system for amplitude recording. Representative tracings are shown in Fig.22.



This figure shows simultaneously obtained tracings during a control state, during pentobarbital intoxication (40 mg/kg) and during an isoproterenol infusion 0.50 µg/kg/min. The close approximation of the peak left ventricular pressures is evident. It is obvious that the changes in LVmax dp/dt recorded by the two systems were proportional to each other in stoichiometric fashion.

The composite data for one of these experiments is given in Table I (Expt. No.6/81, 22/10/68, Dog W.146). Each reading supplied represents the mean of 12 observations during each period recorded. The statistical regression analysis is indicated.

TABLE I.

dp/dt output (mm) Catheter-manometer (X)	dp/dt output (mm) Micromanometer (Y)	(XY)
25.4	20.1	510.54
25.8	18.4	474.72
28.8	21.3	613.44
31.7	22.7	719.59
34.5	27.5	948.75
39.8	33.7	1341.26
42.8	37.5	1605.00
44.0	41.0	1804.00
45.3	40.6	1839.18
$\sum X = 318.1$	$\sum Y = 262.8$	$\sum XY = 9856.48$
$N = 9$	$N = 9$	
$\bar{X} = 35.34$	$\bar{Y} = 29.20$	
$\sum X^2 = 11739.3$	$\sum Y^2 = 8339.1$	
$(\sum X)^2 = 101187.6$	$(\sum Y)^2 = 69063.8$	
$\frac{(\sum X)^2}{N} = 11243.06$	$\frac{(\sum Y)^2}{N} = 7673.76$	$\frac{(\sum X)(\sum Y)}{N} = 9288.52$
$\sum x^2 = 496.08$	$\sum y^2$	

$$b = \frac{\Sigma xy}{\Sigma x^2} = \frac{576.96}{496.28} = 1.144$$

$$\hat{Y} = \bar{Y} + b\,(X-\bar{X}) = 29.20 + 1.144\,X - (35.34 \times 1.144) = 1.144X - 11.2$$

$$\sum d_{xy}^{\,2} = \Sigma y^2 - \frac{(\Sigma xy)^2}{\Sigma x^2} = 665.34 - 649.993 = 15.347$$

$$S_{xy}^{\,2} = \frac{\sum d_{xy}^{\,2}}{N-2} = 2.192; \quad S = 1.48$$

$$S_b = \frac{S_{xy}}{\sqrt{\Sigma x^2}} = \frac{1.48}{22.27} = 0.066; \quad t = \frac{b}{S_b} = \frac{1.144}{0.066} = 17.33 \ (p<0.001)$$

Source of variation	Degrees of freedom	Sum of squares	Mean Square
The mean	1	$\frac{(\Sigma Y)^2}{N} = 7673.76$	
Regression	1	$\frac{(\Sigma xy)^2}{\Sigma x^2} = 649.99$	
Deviations from regression	3	$\sum d_{xy}^{\,2} = 15.35$	5.116
Total	5	$\Sigma y^2 = 8339.10$	

The outputs of the two systems have a related linear regression to a highly significant degree ($p<0.001$) over the range of 5,000 to 11,000 mm Hg/sec, attained in this experiment. The values obtained in this experiment are plotted in Fig.23 on Page 83.

Similar highly significant regressions were obtained in the other experiments ($p<0.001$ in each instance).

These findings demonstrate that the catheter-manometer system used in these experiments gave the same degree of fidelity of measurement as the micromanometer system, particularly of the important measure of LVmax dp/dt, over a wide range. The range of rates concerned was 50 to 200 beats per minute and the range of LVmax dp/dt was 600 to 12,000 mm Hg/sec. These findings are consonant with the views and findings of Knopp et al[90] and Murphy et al.[111]

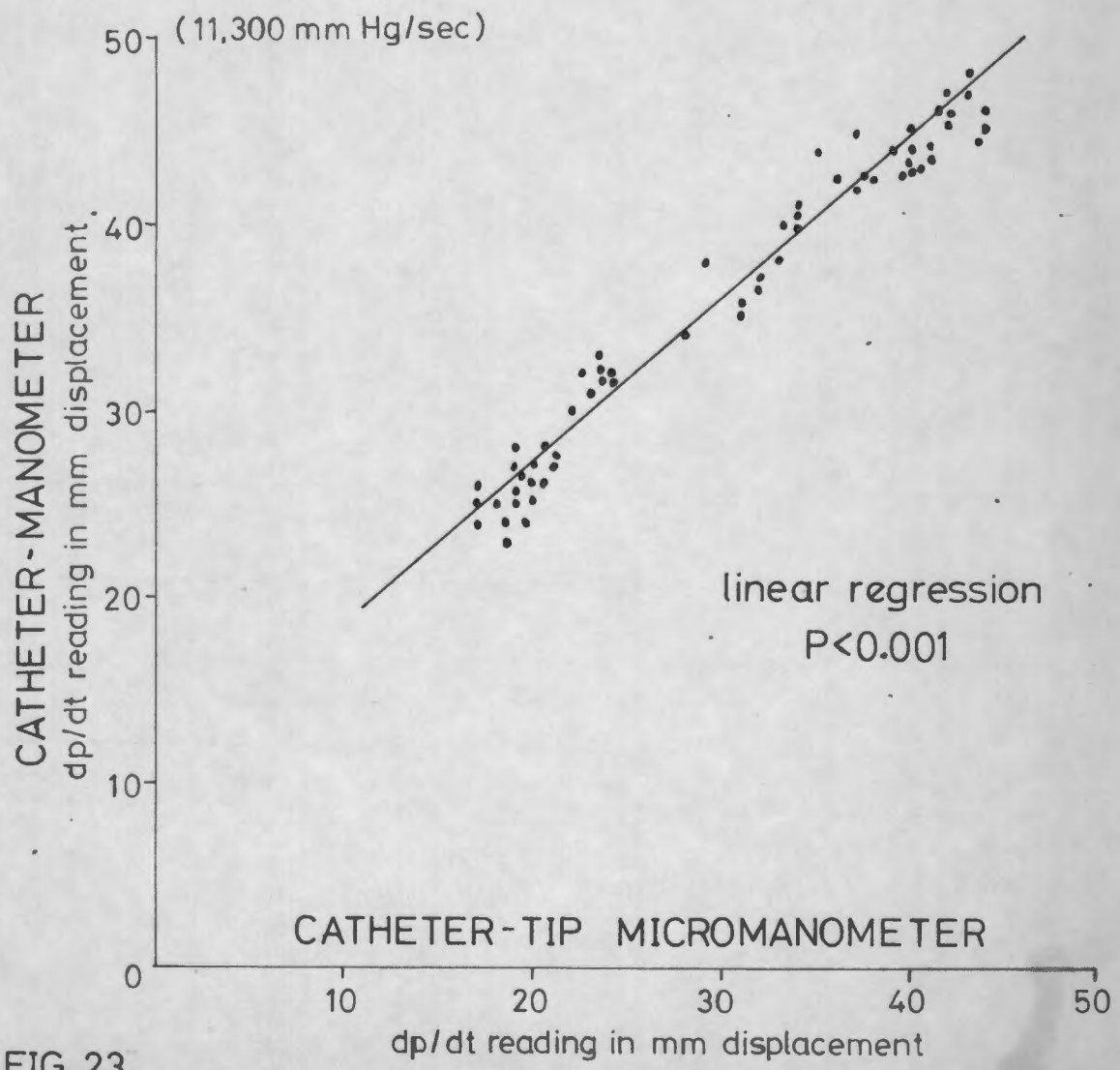


FIG. 23

5. RESULTS.

1. MYOCARDIAL CONTRACTILITY RELATED TO THYROID STATUS:

25 normal and 17 dogs treated with I^{131} between 6 and 50 weeks previously (mean 17.5 weeks), were studied. Six observations were also obtained in 4 animals treated with I^{131} more than 2 years previously (referred to as the myxoedematous group). These latter animals had sluggish movements, a coarse skin, sparse hair growth and lard-like subcutaneous tissues - all the characteristic features of myxoedema in the dog.

(a) Serum Total Cholesterol:

The serum cholesterol levels in 18 normal animals, for 19 observations in the hypothyroid group and for 6 observations in the myxoedematous group are indicated in Table II. Since the animals were not paired, an analysis of variance rather than a "t-test" is performed, comparing the various groups as units. The I^{131}-treated groups could have been treated as a paired group with each dog serving as its own control, but since a few samples were lost, the analysis of variance is conducted instead.

TABLE II.

TOTAL SERUM CHOLESTEROL (mg/100 ml).

Normal	Hypothyroid	Myxoedematous
79	277	543
141	398	320
95	131	327
137	143	423
117	190	474
76	432	586
154	429	
130	366	

	Normal	Hypothyroid	Myxoedematous
	121	637	
	143	391	
	140	483	
	178	309	
	113	325	
	141	400	
	86	310	
	143	222	
	119	468	
	148	425	
		230	
ΣX	2261	6566	2673
N	18	19	6
$\bar{X}$	125.61	345.57	445.5
ΣX^2	296,931	2,564,442	1,251,179
$(\Sigma X)^2$	5,112,121	43,112,356	7,144,929
$\frac{(\Sigma X)^2}{N}$	284,006.7	2,269,071.4	1,190,821
Σx^2	12,925	295,371	60,357
S.E.	6.50	29.44	44.85

Comparison of Normal versus Hypothyroid groups

	Normal	Hypothyroid
$\bar{X}$	125.61	345.57
d.f.	17	18
Σx^2	12,925	295,371

$$\text{Pooled variance} = \frac{12925 + 295371}{17 + 18} = 8808.45 \text{ (35 d.f.)}$$

$$S_{\bar{X}_1 - \bar{X}_2} = \sqrt{8808.45(1/18 + 1/19)}$$

$$= \sqrt{952.193}$$

$$= 30.85$$

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S_{\bar{X}_1 - \bar{X}_2}} = \frac{345.57 - 125.61}{30.85} = \frac{219.96}{30.85} = 7.13$$

for 35 d.f., $t = 7.13$, $P < 0.001$

Similarly a comparison between the hypothyroid and myxoedematous groups yielded a t value of 1.72. For 23 d.f. this yields a P value of < 0.10 and this is not regarded as significant. The dogs treated with I^{131} therefore had serum cholesterol levels elevated above normal to a highly significant degree. No difference in serum cholesterol levels was apparent between the dogs treated with I^{131} between 6 and 50 weeks and those more than 2 years previously.

Serum cholesterol levels were not consistently measured in the dogs treated with thyroxine; several levels had to be discarded because of interference with the colour reaction by indocyanine green which had been administered for the performance of cardiac output determinations and the data were therefore not analysed.

(b) Body Weight:

The values for the 17 animals before and after treatment with I^{131} are given in Table III. The mean time of observation after I^{131} treatment was 17.5 weeks. The data for the myxoedematous group are included in the same table

separately since each dog acted as its own control. The statistical method used is Student's "t-test" for paired samples.

TABLE III.

BODY WEIGHTS (Kg.)

Hypothyroid Group			Myxoedematous Group		
Before I^{131}	After I^{131}	Δ	Before I^{131}	After I^{131}	Δ
10.9	15.0	+4.1	16.4	25.0	+8.6
16.1	17.5	+1.4	16.8	25.4	+8.6
10.0	18.0	+8.0	20.9	25.8	+4.9
15.0	16.0	+1.0	22.2	30.8	+8.6
11.0	13.0	+2.0			
10.0	13.0	+3.0			
13.2	16.0	+2.8			
10.5	13.5	+3.0			
12.1	14.5	+2.4			
12.3	13.6	+1.3			
11.2	14.0	+2.8			
13.6	17.7	+4.1			
11.8	14.5	+2.7			
13.2	15.9	+2.7			
13.2	13.6	+0.4			
12.3	18.8	+6.5			
10.9	13.8	+2.9			
ΣX 207.30	258.40	51.1	76.3	107.0	30.70
N 17	17	17	4	4	4
$\bar{X}$ 12.19	15.2	3.006	19.07	26.75	7.67

Sum of squares of differences = 211.11

$$S^2_d = \frac{\sum d^2 - \frac{(\sum d)^2}{N}}{N-1}$$

$$= \frac{211.11 - \frac{(51.1)^2}{17}}{16}$$

$$= \frac{57.509}{16}$$

$$= 3.594$$

$$t = \frac{\bar{d}}{\sqrt{\frac{S^2_d}{N}}}$$

$$= \frac{3.006}{0.4597}$$

$$= 6.538$$

for 16 degrees of freedom $p < 0.001$

Sum of squares of differences = 245.89

$$S^2_d = \frac{\sum d^2 - \frac{(\sum d)^2}{N}}{N-1}$$

$$= \frac{245.89 - \frac{(30.7)^2}{4}}{3}$$

$$= \frac{10.27}{3}$$

$$= 3.423$$

$$t = \frac{\bar{d}}{\sqrt{\frac{S^2_d}{N}}}$$

$$= \frac{7.67}{0.925}$$

$$= 8.291$$

for 3 degrees of freedom $p < 0.005$

Both the hypothyroid and the myxoedematous dogs had gained weight to a highly significant degree since receiving I^{131}. All dogs had food available at all times and could eat if they so wished. In contrast 12 of the normal dogs were kept under the same circumstances for more than six months. There was no weight gain in these dogs.

The weight data for the group of animals receiving daily injections of thyroxine are supplied in Table IV. Since each dog acted as its own control the statistical method used was as

indicated, a Student's "t-test" for paired values. Data are indicated for control readings, 1 week of treatment and 3 weeks of treatment.

TABLE IV.

BODY WEIGHTS (Kg).

Control Weight	1 week T4	Δ	3 weeks T4	Δ
13.6	12.3	-1.3	11.8	-0.5
11.9	10.5	-1.4	dead	
11.8	10.9	-0.9	dead	
12.3	11.8	-0.5	dead	
12.4	11.9	-0.5	11.3	-0.6
11.4	11.3	-0.1	10.6	-0.7
10.7	10.4	-0.3	9.9	-0.5
10.6	10.0	-0.6	9.2	-0.8
$\sum X$ 94.7	89.10	5.6	52.8	3.10
N 8	8	8	5	5
$\bar{X}$ 11.84	11.14	0.70	10.56	0.62
$\sum \Delta^2$		5.42		1.99

$$s^2_d = \frac{5.42 - \frac{(5.6)^2}{8}}{7}$$

$$= 0.214$$

$$t = \frac{0.70}{\sqrt{\frac{0.214}{8}}}$$

$$= \frac{0.70}{0.1634}$$

$$= 4.28$$

for 7 degrees of freedom $p < 0.005$

$$s^2_d = \frac{1.99 - \frac{(3.1)^2}{5}}{4}$$

$$= 0.017$$

$$t = \frac{0.62}{\sqrt{\frac{0.017}{8}}}$$

$$= \frac{0.62}{0.0459}$$

$$= 13.53$$

for 4 degrees of freedom $p < 0.001$

Three of the dogs studied at one week died before the study at 3 weeks and the appropriate statistical changes were made. It is evident that treatment with thyroxine 0.1 mg/kg daily, caused a significant loss of weight after one week and a further significant loss after two further weeks of treatment.

(c) LVmax dp/dt:

Recordings were obtained in all the animals. The data are arranged as for serum cholesterol and body weight with separate analysis of the group receiving thyroxine since these animals served as their own controls. The values are indicated in Table V(a), for the I^{131} treatment experiments and Table V(b) for the thyroxine treatment experiments. c.f. page 91.

TABLE V(a).

LVmax dp/dt (mm Hg/sec).

Normal	Hypothyroid	Myxoedematous
4640	3200	2040
4640	3300	2210
4370	3300	1970
4420	3340	2100
4120	3430	2480
4800	2930	2260
4160	3780	
5100	2600	
5000	3710	
5910	2800	
4930	3740	
4940	3300	
6100	3660	
4360	3770	
5100	2760	
6350	2460	
6490	2950	
6400		
5000		
6100		
5100		
6400		
6400		
5660		
7200		

ΣX	133,690	55,030	13,060
N	25	17	6
$\bar{X}$	5,347	3,237	2,176
ΣX^2	7,333,069 *	1,810,357 *	285,946 *
$(\Sigma X)^2$	178,730,161	30,283,009 *	1,705,636 *
$\frac{(\Sigma X)^2}{N}$	7,149,206 *	1,781,353 *	284,273 *
Σx^2	183,862 *	29,003 *	1,673 *
S.E.	175.0	103.3	74.7

(* given X 10^{-2})

(i) Comparison of Normal and Hypothyroid Groups (Analysis of variance).

	Normal	Hypothyroid
$\bar{X}$	5347.6	3237.0
$\sum x^2 - (X10^{-2})$	183,862	29,003
d.f.	24	16

Pooled variance $= \frac{183862 + 29003}{24 + 16} = 5321.6$ (for 40 d.f.)

$$S_{\bar{x}_1 - \bar{x}_2} = \sqrt{S^2 \left(\frac{1}{N_1} + \frac{1}{N_2}\right)}$$

$$= \sqrt{5321.6 \left(\frac{1}{25} + \frac{1}{17}\right)}$$

$$= \sqrt{525.774}$$

$$= 22.92$$

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S_{\bar{x}_1 - \bar{x}_2}} = \frac{534.76 - 323.7}{22.92} = \frac{211.06}{22.92}$$

$$= 9.208$$

for 40 d.f., $P < 0.001$

(ii) Comparison of Hypothyroid and Myxoedematous Groups (Analysis of Variance.)

	Hypothyroid	Myxoedematous
$\bar{X}$	3237.0	2176.0
$\sum x^2 (x10^{-2})$	29003	1673
d.f.	16	5

Pooled variance = $\frac{29003 + 1673}{16 + 5}$ = 1460.78 (for 21 d.f.)

$$S_{\bar{x}_1 - \bar{x}_2} = \sqrt{1460.78 \left(\frac{1}{17} + \frac{1}{6}\right)}$$

$$= \sqrt{329.259}$$

$$= 18.14$$

$$t = \frac{323.7 - 217.6}{18.14} = \frac{106.1}{18.14} = 5.84$$

for 21 d.f. $P < 0.001$

TABLE V(b).

LVmax dp/dt (mm Hg/sec).

	Control.	1 week Thyroxine.	1 week Thyroxine.	3 weeks Thyroxine.
	5,000	11,420	11,420	11,750
	6,100	7,920	-	-
	5,100	11,200	-	-
	6,350	10,900	-	-
	6,400	9,400	9,400	13,100
	6,400	8,830	8,830	12,670
	5,660	11,430	11,430	10,000
	7,200	10,970	10,970	10,700
ΣX	48,210	82,070	52,050	58,220
N	8	8	5	5
$\bar{X}$	6,026	10,258	10,410	11,644
* ΣX^2	2,943,381	8,547,075	5,477,311	6,846,914
* $(\Sigma X)^2$	23,242,041	67,354,849	27,092,025	33,895,684
* $\frac{(\Sigma X)^2}{N}$	2,905,255	8,419,356	5,418,405	6,779,137
* Σx^2	38,126	127,719	58,906	67,777
S.E.	260.9	477.6	606.7	650.8

(* values given X 10^{-2})

(i) Comparison of Control and 1 week treatment periods (paired t-test).

Mean difference = 423.25

Sum of squares of differences = 1,648,520

$$s_d^2 = \frac{\sum d^2 - \frac{(\sum d)^2}{N}}{N-1}$$

$$= \frac{1,648,520 - 1,433,124.5}{7}$$

$$= \frac{215,395.5}{7}$$

$$= 30,770.7$$

$$t = \frac{1025.8 - 602.6}{\sqrt{\frac{30,770.7}{8}}}$$

$$= \frac{423.3}{62.01} = 6,824$$

= for 7 d.f. $P < 0.001$

(ii) Comparison between one and three week treatment periods (paried t-test).

Mean difference = 123.4

Sum of squares of differences = 3066.23

$$s_d^2 = \frac{306,623 - 76,137.8}{4}$$

$$= \frac{230,485.2}{4}$$

$$= 57,621.3$$

$$t = \frac{1164.4 - 1041}{\sqrt{\frac{57,621.3}{5}}}$$

$$= \frac{123.4}{\sqrt{11524.26}}$$

$$= \frac{123.4}{107.35} = 1.49$$

for 5 d.f. $P > 0.5$

The data for the normal, hypothyroid and myxoedematous groups are shown in Fig.24. The results obtained in the dogs treated with thyroxine are included for comparison.

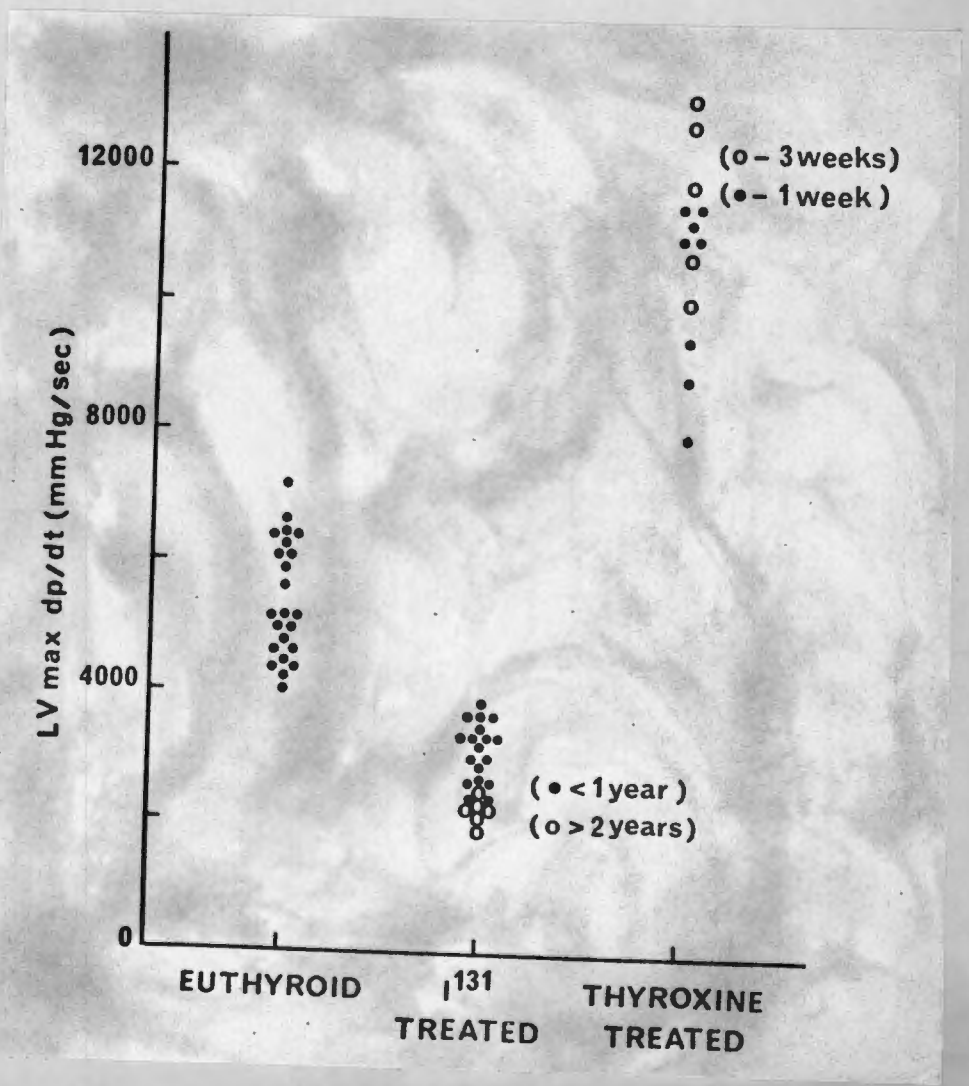


FIG. 24

These results indicate that both I^{131} and thyroxine induced highly significant deviations in LVmax dp/dt away from normal. I^{131} treatment resulted in a highly significant reduction in LVmax dp/dt in the hypothyroid group studied at a mean of 17.5 weeks after treatment, with the myxoedematous group studied between two and two-and-a-half years undergoing a further highly significant reduction.

Thyroxine treatment, in the dosage used in these experiments (0.1 mg/kg/day) caused a highly significant elevation of LVmax dp/dt in each dog. The significance of this is heightened by the fact that each dog acted as its own control. Further treatment for an additional two weeks did not increase the levels to a significant degree. Inspection of the data reveals, however, that the animals that responded maximally in one week did not undergo any further increase whereas the animals that showed a more modest increase at one week had larger increases during the subsequent two weeks of treatment.

(d) Other Haemodynamic Measurements:

The analyses of the values obtained for heart rate, cardiac index, stroke volume index, mean aortic pressure, LVEDP, ejection time, mean systolic ejection rate, left ventricular stroke work index, left ventricular stroke power index, peripheral resistance index and right ventricular max dp/dt are indicated in summary form in Table VI; the values of LVmax dp/dt are included for the sake of completeness. The actual values are indicated in Table 2 in the Appendix. In this table the mean values and the standard errors of the means are indicated with the levels of significance of the deviations. The table is again divided into sections

(a) and (b) with section (a) containing the data for the experiments involving treatment with I^{131} and section (b) the data for the experiments with thyroxine.

TABLE VI(a).

Number of dogs	Normal 23	Hypothyroid 17	Δ from normal	Myx-oedematous 6	Δ from normal	Δ from hypo-thyroid
LVmax dp/dt (mm Hg/sec)	5347±175	3237±103	P<0·001	2176±75	P<0·001	P<0·001
Heart rate (per min)	78±2.5	67±3.6	P<0·025	47±3.4	P<0·001	P<0·01
Cardiac index (ml/min/m^2)	3614±113	2135±152	P<0·001	1058±35	P<0·001	P<0·001
S.V.I. (ml/m^2)	46.5+1.7	32.8±2.3	P<0·001	22.8±1.2	P<0·001	P<0·025
L.V.S.P. (mm Hg)	156±2.9	137±4.8	P<0·001	115±8.3	P<0·001	P<0·05
M.A.P. (mm Hg)	108±2.5	104±3.4	N.S.	87±6.6	P<0·005	P<0·025
LVEDP (mm Hg)	9.02±0.67	9.94±0.76	N.S.	10.18±0.78	N.S.	N.S.
Ejection time (msec)	204±2.7	213±3.5	P<0·10	228±5.4	P<0·001	P<0·05
MSER (ml/m^2/sec)	228±8.2	153±9.7	P<0·001	99±4.5	P<0·001	P<0·005
LVSWI (gm/m)	62.3±3.3	41.4±3.4	P<0·001	22.2±1.6	P<0·001	P<0·005
LVSPI (gm/m/sec)	306±15.8	193±14.9	P<0·001	97±6.3	P<0·001	P<0·005
P.R.I. (dyn.sec.$cm^{-5}m^{-2}$)	2430±77	4160±276	P<0·001	6555±48	P<0·001	P<0·001
RVmax dp/dt (mm Hg/sec)	1342±94	1025±88	P<0·025	515±95	P<0·025	N.S.

TABLE VI(b).

	No.	Control period	1 week T4	3 weeks T4	C. + 1 wk. T4	1-3 wks T4	C-3 wks T4
Body weights (kg)	8	11.84±0.34	11.14±0.29		P<.005		
	5	11.74±0.56	11.18±0.44	10.56±0.47		P<.001	P<.005
LVmax dp/dt (mm Hg/sec)	8	6026±261	10258±477		P<.001		
	5	6132±373	10410±607	11644±651		N.S.	P<.005
Heart rate (per min)	8	80±4.8	112±3.9		P<.005		
	5	79±7.2	110±4.6	143±19		N.S.	P<.05
Cardiac index ($ml/min/m^2$)	8	3739±188	5080±412		P<.001		
	5	3957±232	5466±608	5502±560		N.S.	N.S.
S.V.I. (ml/m^2)	8	47.1±3.2	45.5±3.8		N.S.		
	5	51.2±3.9	49.6±5.1	40.1±4.5		N.S.	N.S.
M.A.P. (mm Hg)	8	110±3.5	118±3.9		P<.05		
	5	110±5.5	116±5.7	109±5.4		P<.05	N.S.
LVEDP (mm Hg)	8	8.52±1.28	5.02±0.57		N.S.		
	5	8.94±2.07	4.80±0.70	4.44±0.72		N.S.	N.S.
Ejection time (msec)	8	204±2.6	176±3.7		P<.001		
	5	207±3.7	176±5.1	154±9.1		N.S.	P<.025
M.S.E.R. ($ml/m^2/sec$)	8	230±15.4	259±21.4		N.S.		
	5	248±20.4	284±28.1	257±43.1		N.S.	N.S.
LVSWI (gm/m)	8	65±5.2	68.6±4.5		N.S.		
	5	70±7.1	73.6±5.5	58.3±8.9		N.S.	N.S.
LVSPI (gm/m/sec)	8	319±25.8	393±25.7		P<.025		
	5	342±36.7	423±30.7	373±46.5		N.S.	N.S.
P.R.I. ($dyn.sec/cm^5m^2$)	8	2385±126	1980±234		N.S.		
	5	2242±136	1856±378	1644±135		N.S.	P<.05
RVmax dp/dt (mm Hg/sec)	8	1471±139	2559±263		P<.01		
	5	1490±225	2662±375	2634±256		N.S.	P<.005

In the dogs treated with I^{131} a significant change occurred not only in LVmax dp/dt as indicated previously, but heart rate was also reduced to a highly significant degree with a further significant reduction apparent in the myxoedematous group in comparison with the hypothyroid group. Associated with these changes were parallel alterations in cardiac index, stroke volume index and ejection time. Peak left ventricular systolic pressure was reduced to a highly significant degree in comparing normal v.s. hypothyroid and hypothyroid v.s. myxoedematous groups. Aortic mean pressure was, however, not different between the normal and hypothyroid groups, but the myxoedematous groups had pressures highly significantly less than normal and significantly less than the hypothyroid group. LVEDP was not significantly different in the hypothyroid and myxoedematous groups in comparison with the normal group. The mean systolic ejection rate was highly significantly reduced from normal to hypothyroid and further from hypothyroid to myxoedematous groups, as were both left ventricular stroke work and power indices. A reduction in myocardial contractility of the left ventricle is therefore reflected also by these indices of contractility in confirmation of the alterations encountered in LVmax dp/dt.

Right ventricular max dp/dt was also measured in all animals. Both the hypothyroid and myxoedematous groups had significantly reduced values, but these two groups were not significantly different from each other.

Treatment with thyroxine had the opposite effect. In Table VI(b) the summary analyses are given. Analyses for each measure were conducted for the 8 animals studied during the control and 1 week periods; three animals died subsequently and

the values obtained for the remaining five animals were extracted from these periods for comparison with the three-week treatment period. In this fashion each dog acted rigidly as its own control.

In addition to the increases in LVmax dp/dt already outlined, heart rate, cardiac index and ejection times were altered significantly between control period and one-week treatment period. Further significant changes between one-week and three-weeks could not be demonstrated; this may be attributed to the fact that fewer animals entered into this analysis with consequently much larger variances. The stroke volume index remained unaltered; although cardiac index increased to a highly significant degree, this was accomplished mainly by an increase in heart rate. The findings with reference to mean systolic ejection rate and stroke work index reflected this as well. Left ventricular stroke power index was, however, increased significantly, reflecting the highly significant reduction in ejection time observed. Caution has to be exercised in regarding this as a true increase of contractility, since such alterations in ventricular ejection are compatible with a mere increase in heart rate (which has with it an autoregulatory increase in contractility[107,112].

In this group of dogs a highly significant increase in RVmax dp/dt could be shown between the control and one-week and control and three-week treatment periods.

Peripheral resistance index was not significantly altered after one week of treatment; this was due to a significant increase in mean aortic pressure that accompanied the large increase in flow. With continued treatment for the second two

weeks a highly significant alteration in flow/pressure relations occurred. The cardiac index continued to rise, although a P value < 0.10 only could be demonstrated (because of a reduced number of animals and consequently higher variance), while the mean arterial pressure underwent a significant reduction yielding a significant reduction in peripheral resistance.

(e) Conclusions:

It is possible to show that I^{131} treatment and thyroxine treatment induce changes in myocardial contractility in opposite directions in normal animals. The evidence for hypothyrodism was an increase in body weight and total serum cholesterol, while the animals on thyroxine, in addition to development of significant tachycardia, all lost weight to a significant extent. The changes in myocardial contractility can be demonstrated independently in both ventricles by measurement of the peak rate of pressure development, reflecting the force-velocity relations operating in the ventricles, and were shown for the left ventricle additionally by mechanisms utilizing the Frank-Starling principle and manifested as changes in output, work and power. In these groups of animals myocardial contractility can be correlated directly with thyroid status.

2. RELATIVE CONTRIBUTIONS OF HEART RATE AND DIRECT EFFECT OF HORMONE TO CONTRACTILITY CHANGES.

Accompanying the changes in myocardial contractility with the alterations in thyroid status were, however, significant changes in heart rate. The next phase in these investigations was directed towards assessment of the relative contributions of the autoregulatory alterations in contractility related to the changes in heart rate and the direct effect of the hormone.

This was studied in the animals that had undergone an increase in myocardial contractility. An attempt was made at increasing the heart rate of the hypothyroid animals by atrial pacing to the range of normal, but this was not possible because of the unexpected high degree of A-V block encountered during artificial atrial pacing at increasing heart rates.

Although these experiments were performed in all 8 animals receiving thyroxine, records suitable for analysis were obtained from 6 dogs only; the other records were rejected because of the occurrence of A-V block to a significant degree at relatively slow rates in one dog and in the other animal the rate at which pacing was initiated was so high that a curve with a satisfactory number of points on it could not be obtained. In each animal a rate-LVmax dp/dt curve was obtained while in the control state and again after treatment with thyroxine. The detailed analysis of the findings in one animal, typical of the group as a whole will be shown, and the data for the rest of the animals will be shown in summary form. All the animals behaved similarly, except one animal that had a high initial rate on one occasion - this was a particularly excitable dog and a high sympathetic tone may have contributed to his atypical findings.

TABLE VII.

Dog G.495: Experiment No. 8/82, 16/8/68, 23/8/68, 6/9/68.

(i) Control state:

Rate: (X)	LVmax dp/dt (Y)	(XY)
113	6720	759,360
122	7030	857,660
137	7620	1,043,940
151	7560	1,141,560
171	7570	1,294,470
193	8200	1,582,600

N = 6

$\sum X$	887	$\sum Y$	44700	$\sum XY$	6,679,590
$\bar{X}$	147.8	$\bar{Y}$	7450		
$\sum x^2$	135,713	$\sum Y^2$	33,342,200	$\frac{(\sum X)(\sum Y)}{N}$	6,608,150
$\frac{(\sum X)^2}{N}$	131,128	$\frac{(\sum Y)^2}{N}$	333,015,000		
$\sum x^2$	4585	$\sum y^2$	1,327,200.	$\sum xy$	71,440

$$b = \frac{\sum xy}{\sum x^2} = \frac{71440}{4585} = 15.58$$

$$\hat{Y} = \bar{Y} + b(X-\bar{X}) = 7450 + 15.58X - 2303 = 15.58X + 5147$$

$$\sum d_{xy}^2 = \sum y^2 - \frac{(\sum xy)^2}{\sum x^2} = 1,327,200 - 1,113,100 = 214,100$$

$$S_{xy}^2 = \frac{\sum d_{xy}^2}{N-2} = \frac{214,100}{4} = 53,525$$

$$S_{xy} = 231.3; \quad S_b = \frac{S_{xy}}{\sqrt{\sum x^2}} = \frac{231.3}{67.7} = 3.416$$

$$t = \frac{b}{S_b} = \frac{15.58}{3.416} = 4.516 \ (P < 0.025)$$

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
The mean	1	$\frac{(\Sigma Y)^2}{N}$ = 333,015,000	
Regression	1	$\frac{(\Sigma xy)^2}{\Sigma x^2}$ = 1,113,100	
Deviations from regression	4	$\sum d^2_{xy}$ = 214,100	53,525
TOTAL	6	$\sum Y^2$ = 334,342,200	

For the following periods, after one week and after three weeks of thyroxine treatment, the procedure followed to calculate the regressions and their significance, was identical to that above.

(ii) After 1 week Thyroxine

Rate	LVmax dp/dt
113	7,780
117	8,040
136	8,680
148	9,300
180	10,350
190	10,030
193	9,980

$b = 28.98$

$\hat{Y} = 28.98X + 4708$

$t = 7.98$ $(P<0.001)$

(iii) After 3 weeks Thyroxine

Rate	LVmax dp/dt
130	11,570
139	12,900
150	13,440
157	13,640
166	13,660
187	14,180

$b = 39.74$

$\hat{Y} = 39.74X + 7079$

$t = 3.75$ $(P<0.025)$

The procedures followed in the other animals were identical to those outlined for the animal above. The collected data are presented in Table VIII. For all animals the LVmax dp/dt at rate 140 was obtained for analysis of statistical significance in both slope and elevation. These are indicated as well.

TABLE VIII.

Dog No.	Coefficient of regression			Y intercept			LVmax dp/dt at rate 140		
	C.	1 wk.	3 wks.	C.	1 wk.	3 wks.	C.	1 wk.	3 wk
G.495	15.58	28.98	39.74	5147	4708	7079	7300	8700	1260
G.500	28.16	30.16	48.00	4088	4379	5267	8000	8600	1210
G.446	20.13	44.65	-	4580	4225	-	7400	10500	-
F.773	10.14	24.27	-	4187	5390	-	5600	8800	-
C.493	47.68	37.34	-	170	3031	-	6800	8200	-
G.428	17.11	22.26	-	3440	8091	-	5800	11200	-
Mean	23.13	31.27	43.87	3602	4971	6173	6816	9333	1235

A "t test" for paired values was then performed on the values occurring at rate 140 during the control and one-week treatment period. Data for 3 weeks were not included because satisfactory curves could be obtained in two animals only. The "t test" revealed a significant elevation in the LVmax dp/dt occurring at the same rate (140) after one week of thyroxine treatment ($P < 0.025$). A graphic illustration is provided in Fig.25. This reveals the initial resting value for all the dogs treated with thyroxine plotted against a curve obtained from the mean values for coefficient of regression and intercept on the Y axis for all the animals in which artificial pacing was performed. The 95% confidence limits of this curve are indicated. It is seen that in every instance the point, the co-ordinates representing the LVmax dp/dt at that particular rate, falls well above the area in which this could have been contributed to by rate alone.

It is furthermore noted that all animals, except one, showed progressive increments in the coefficients of regression. Because the numbers were so small, statistical analysis was not attempted.

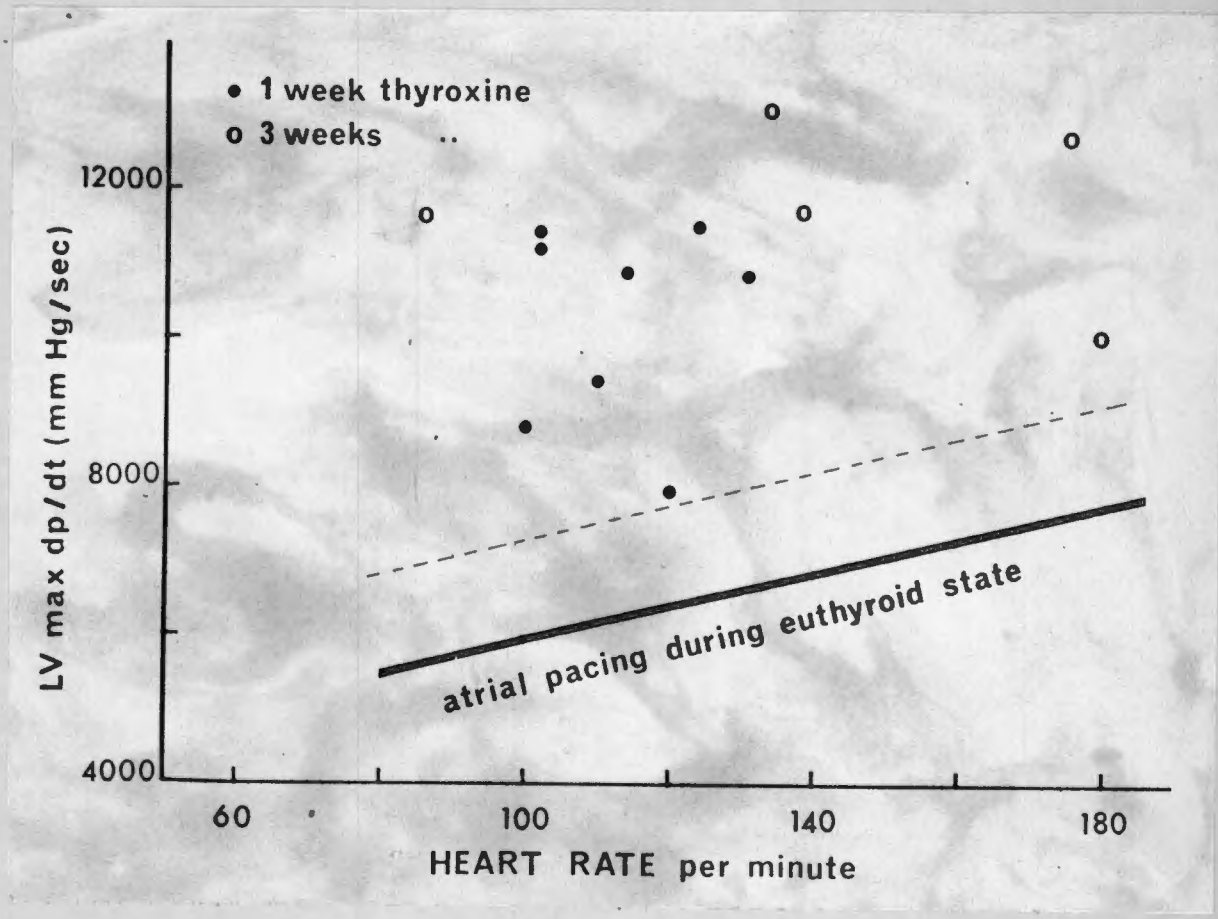


FIG. 25

Conclusion:

The administration of thyroxine to these dogs resulted in an increase in LVmax dp/dt as well as heart rate. When the heart rate was elevated over a wide range of rates by artificial atrial pacing, the levels for LVmax dp/dt were significantly higher at all levels of heart rate after thyroxine. This is interpreted as indicating that the treatment with thyroid hormone induced an elevation of myocardial contractility greater than could be attributed to the increase associated merely with the increase in rate. A change also occurred in the coefficients of linear regression which probably indicates an alteration in the rate - contractility autoregulating mechanism, but the

contribution of this to the increased contractility appeared relatively insignificant.

3. INFLUENCE OF THYROID STATUS ON RESPONSES TO BETA-ADRENERGIC STIMULATION.

Following the demonstration that the resting haemodynamic profile of the intact conscious dog could be correlated with its thyroid status, with particular reference to indices of myocardial contractility, it was thought relevant to investigate the behaviour of the myocardium under the influence of beta-adrenergic stress. The agent isoproterenol, administered by 12 minute infusions at various doses was used. Dose-response curves were obtained in normal, hypothyroid, myxoedematous groups and in the thyroxine-treated animals during the control state, after one week and again after three weeks of treatment. Since each dog acted as its own control in the thyroxine-treatment experiments, these data are analysed separately.

The statistical approach was a two-way analysis of variance of the response profile for each group of dogs. The responses were partitioned further into the various components of treatment effects and regression, linear, quadratic and cubic. An inter-group comparison became more feasible with such a detailed within-group vertical and horizontal analysis.

The data for LVmax dp/dt in these groups is presented in Table IX; the statistical method followed is outlined. The data for the other haemodynamic observations are presented in Table X while the details omitted here are contained in the Appendix Table 3.

Since LVmax dp/dt is calculated to the nearest 10 mm Hg/sec

the statistical manipulations are all performed coded at 10^{-1}; hence values are indicated $\times 10^{-1}$.

TABLE IX.

(a) LVmax dp/dt during isoproterenol infusions in Normal Dogs.

	Isoproterenol dose (µg/kg/min)		
Baseline	0.05	0.10	0.20
464	516	532	663
464	654	748	916
437	540	654	680
480	579	729	818
416	535	552	600
510	574	661	775
500	505	547	650
591	608	680	794
493	556	572	592
494	530	547	565
610	775	880	936
436	493	543	612
510	745	823	888
635	663	668	731
649	731	780	884
640	880	837	888
500	505	547	650
610	775	880	936
510	745	823	888
640	731	780	884
640	880	837	888
566	590	625	644
720	796	824	1005
$\sum X$ 12515	14906	16069	17887
N 23	23	23	23
$\bar{X}$ 544.13	648.09	698.65	777.70
$\sum x^2$ 6,967,961	9,999,420	11,562,591	14,318,845
$\sum x^2$ 158,168	339,036	335,949	408,203

The components of regression for the treatment sum of squares were calculated using the following system of weighted coefficients:

Totals	Linear Regression	Quadratic Regression	Cubic Regression
12515	-7	+7	+3
14906	-3	-4	-8
16069	+1	-8	+6
17887	+9	+5	-1
Divisor	3220	3542	2530
Contrast	44,729	-11,136	-3176
Sum Sq.	621,330	35,011	3987
F	214.25	12.07	1.37
P	< 0.001	< 0.005	N.S.

The final analysis of variance therefore was:

Source of variation	Degrees of freedom	Sum of Squares	Mean Square	F.	P.
Total	91	1,901,685			
Error	66	191,400	2,900		
Dogs	22	1,049,957	47,725	16.4	<.001
Treatments	3	660,328	220,109	75.8	<.001
(a) Linear components	1	621,330		214.25	<.001
(b) Quadratic components	1	35,011		12.07	<.005
(c) Cubic components	1	3,987		1.37	N.S.

(b) LVmax dp/dt during isoproterenol infusions in Hypothyroid Dogs.

Baseline	Isoproterenol (µg/kg/min)		
	0.05	0.10	0.20
330	380	431	486
334	382	547	604
343	430	510	536
293	382	386	470
378	502	555	763
260	386	452	572
371	489	556	672
280	334	361	546
374	397	414	469
223	334	423	465
242	421	507	629
ΣX 3428	4437	5142	6212
N 11	11	11	11
$\bar{X}$ 311.64	403.36	467.46	564.73
Σx^2 1,098,648	1,819,231	2,453226	3,600,088
Σx^2 30,358	29,506	49,575	92,002

The components of regression for the treatment sum of squares were calculated using the following system of weighted coefficients:

Totals	Linear Regression	Quadratic Regression	Cubic Regression
3428	-7	+7	+3
4437	-3	-4	-8
5142	+1	-8	+6
6212	+9	+5	-1
Divisor	1540	1694	1210
Contrast	23743	-3828	-572
Sum Sq.	366058	8651	270
F	178.65	4.22	0.13
P	<0.001	<0.05	N.S.

The final analysis of variance for the hypothyroid group therefore was:

Source of variation	Degrees of freedom	Sum of Square	Mean Square	F.	P.
Total	43	576,421			
Error	30	61,472	2,049		
Dogs	10	139,970	13,997	6.83	<.001
Treatments	3	374,979	124,993	61.0	<.001
(a) Linear comp.	1	366,058		178.65	<.001
(b) Quadratic comp.	1	8,651		4.22	<.05
(c) Cubic comp.	1	270		0.13	N.S.

(c) LVmax dp/dt during isoproterenol infusions in myxoedematous dogs:

Baseline	Isoproterenol (μg/kg/min)		
	0.05	0.10	0.20
204	287	320	314
221	248	297	319
197	327	406	598
210	278	359	429
$\sum X$ 832	1140	1382	1660
N 4	4	4	4
$\bar{X}$ 208.0	285.0	345.5	415.0
$\sum x^2$ 173,366	328,086	484,326	742,002
$\sum x^2$ 310	3186	6845	53,102

The components of regression for the treatment sum of squares were calculated using the following system of weighted coefficients:

Totals	Linear regression	Quadratic regression	Cubic regression
832	-7	+7	+3
1140	-3	-4	-8
1382	+1	-8	+6
1660	+9	+5	-1
Divisor	560	616	440
Contrast	7078	-1492	8
Sum of squares	89461	3614	0
F	24.21	0.97	0
P	< 0.001	N.S.	N.S.

The final analysis of variance was:

Source of variation	Degrees of freedom	Sum of Squares	Mean square	F.	P.
Total	15	156,518			
Error	9	33,253	3694.7		
Dogs	3	30,190	10,063	2.72	N.S.
Treatments	3	93,075	31,025	8.39	<0.01
(a) Linear regression	1	89,461		24.21	<0.001
(b) Quadratic regression	1	3614		0.97	N.S.
(c) Cubic regression	1	0		0	N.S.

From these analyses, the linear dose-response curves were calculated:

(a) Normal animals:

Ti	u	$\hat{y}$ (calculated below)
12,515	-7	569.91
14,906	-3	625.47
16,069	+1	681.03
17,887	+9	792.15

$$b = \frac{\text{Contrast}}{\text{Divisor}} = \frac{44,729}{3220} = 13.89$$

$$\hat{y} = \bar{y} + bu.$$

$$\bar{y} = \frac{Ti}{92} = \frac{61,377}{92} = 667.14$$

$$\therefore y = 667.14 + 13.89\ u.$$

u			$\hat{y}$
-7	667.14 - 97.23	=	569.91
-3	667.14 - 41.67	=	625.47
+1	667.14 + 13.89	=	681.03
+9	667.14 + 125.01	=	792.15

To revert from the weighted values to units of isoproterenol:

u	x
-7	0
-3	0.05
+1	0.10
+9	0.20
	0.35

$\bar{x} = 0.0875$

$b' = \frac{8}{0.1} = 80$

$u = b'(X-\bar{X}) = 80x - 7$

Therefore:

$$y = \bar{y} + bu = \bar{y} + b(80X-7) = \bar{y} - 7b + 80\ bX$$
$$= 667.14 - (7 \times 13.89) + (80 \times 13.89X)$$
$$= 569.91 + 1111.20X$$

The equation for the linear slope describing the response of LVmax dp/dt in the normal animals is therefore -

$$Y = 1111.2X + 569.91$$

in terms of units of isoproterenol administered.

The same procedure was followed for the hypothyroid and myxoedematous groups.

For hypothyroid group $Y = 1232.8X + 328.92$

For myxoedematous group $Y = 1011.2X + 224.89$

The dose-response curves were then examined for differences in slope and elevation above the X axis. The error sum of squares for each group was used to calculated a pooled variance.

(a) Comparison of slopes, normal v.s. hypothyroid groups:

$$\text{Pooled variance } (S^2) = \frac{191,400 + 61,472}{66 + 30} = \frac{252,872}{96} = 2634$$

b (normal) = 13.89

with variance $\frac{S^2}{\text{Divisor}} = \frac{2634}{3220} = 0.818$

b (hypothyroid) = 15.41

with variance $\frac{S^2}{\text{Divisor}} = \frac{2634}{1540} = 1.7103$

$$t = \frac{b_1 - b_2}{\sqrt{S^2 \left(\frac{1}{\text{Divisor}_1} + \frac{1}{\text{Divisor}_2} \right)}}$$

$$= \frac{15.41 - 1389}{\sqrt{2634 \left(\frac{1}{3220} + \frac{1}{1540} \right)}} \qquad = \frac{1.52}{\sqrt{2.526}}$$

$$= \frac{1.52}{1.589} = 0.956 \quad \text{for 96 d.f.} \quad P \text{ is not significant.}$$

(b) The slopes of the hypothyroid and myxoedematous groups were similarly compared and no significant differences were obtained.

(c) Comparison of elevation above X axis, normal v.s. hypothyroid groups was obtained by comparing the overall means of each curve, using the pooled variances to obtain an estimate of error.

$\bar{Y}$ (normal) = 667.14

$\bar{Y}$ (hypothyroid) = 436.79

pooled variance (S^2) = 2634

$$t_{(32 \text{ d.f.})} = \frac{\bar{Y}n - \bar{Y}h}{\sqrt{S^2 \left(\frac{1}{N_1} + \frac{1}{N_2} \right)}}$$

$$= \frac{667.14 - 436.79}{\sqrt{2634 \left(\frac{1}{23} + \frac{1}{11} \right)}}$$

$$= \frac{230.35}{18.81} = 12.25$$

for 32 degrees of freedom $P < 0.001$

When similarly compared, the elevation of the hypothyroid and myxoedematous groups were highly significantly different ($P < 0.001$

(d) A comparison was also done, in similar fashion to that for the linear regression component, of the quadratic regression components between normal and hypothyroid groups, but with a "t" value of 0.587, the difference was not significant.

The analyses for heart rate and cardiac index, conducted in similar fashion, are presented in Table X. The data for the variance analyses are presented in summary fashion; an asterisk indicates where a component reached significance. The values for LVmax dp/dt and cardiac index were analysed, coded X 10^{-1}.

TABLE X.

Source of variation	Degrees of freedom N.	H.	M.	Sums of Squares N.	H.	M.
1. LVmax dp/dt						
Total	91	43	15	1,901,685	576,421	156,518
Error	66	30	9	191,400	61,472	33,253
Dogs	22	10	3	1,049,957*	139,970*	30,190
Treatments	3	3	3	660,328*	374,979*	93,075*
Linear component	1	1	1	621,330*	366,058*	89,461*
Quadratic component	1	1	1	35,011*	8,650*	3,614
Cubic component	1	1	1	3,987	270	0
2. Heart rate						
Total	91	43	15	24,229	7,789	959
Error	66	30	9	3,321	1,256	341
Dogs	22	10	3	9,361*	3,411*	209
Treatments	3	3	3	11,547*	3,122*	409
Linear component	1	1	1	11,450*	2,988*	405*
Quadratic component	1	1	1	51	65	0
Cubic component	1	1	1	46	69	4
3. Cardiac index						
Total	91	43	11	1,486,585	415,818	8,280
Error	66	30	6	252,101	73,353	2,094
Dogs	22	10	2	670,195*	254,297*	416
Treatments	3	3	3	564,289*	88,168*	5,770*
Linear component	1	1	1	558,811*	78,886*	2,615*
Quadratic component	1	1	1	965	3,355	1,597
Cubic component	1	1	1	4,513	5,927	1,558

N = Normal H = Hypothyroid M = Myxoedematous

In table XI are indicated the results of the analyses for significant differences between the various groups for LVmax dp/dt, heart rate and cardiac index. The values for the various animals are indicated in Table 3 of the Appendix.

TABLE XI.

	Coefficients of linear regression			Differences (P value)		
	N	H	M	N-H	N-M	H-M
LVmax dp/dt	13.89	15.41	12.63	N.S.	N.S.	N.S.
Heart rate	1.885	1.392	0.85	<.025	<.005	N.S.
Cardiac index	13.17	7.16	2.495	<.005	<.001	N.S.

	Grand means ($\bar{Y}$)			Differences (P value)		
	N	H	M	N-H	N-M	H-M
LVmax dp/dt	6671	4368	3134	<.001	<.001	<.001
Heart rate	90.4	77.5	50.3	<.001	<.001	<.001
Cardiac index	4294	2738	1420	<.001	<.001	<.001

The grand means ($\bar{Y}$) are supplied as measures of vertical displacement.

N = Normal H = Hypothyroid M = Myxoedematous

Thyroxine treatment and responses to isoproterenol.

The data are shown in detail for LVmax dp/dt to demonstrate the marked effect that thyroxine treatment had on these responses. Only the data at one week of treatment are analysed, the responses at 3 weeks were so variable that statistical analysis was not possible. These will therefore be shown in graphical form.

Table XII contains the responses of LVmax dp/dt during isoproterenol infusions in the doses 0 (baseline), 0.05, 0.10 and 0.20 µg/kg/min, first during the control study and again after 1 week of thyroxine treatment (0.1 mg/kg/daily). The data are again handled coded X 10^{-1}.

TABLE XII.

Dog No.	Control study (Dosages of isoproterenol (µg/kg/min) 0	0.05	0.10	0.20	1 week thyroxine 0	0.05	0.10	0.20
1	500	505	547	650	1142	1310	1169	1160
2	610	775	880	936	792	1061	1160	1029
3	510	745	823	880	1120	1475	1534	1575
4	640	731	780	884	940	1274	1023	1116
5	640	880	837	888	883	1093	1284	1392
6	566	590	625	644	1143	1316	1385	1334
7	720	796	824	1005	1097	1236	1269	1304
$\sum X$	4186	5022	5316	5887	7117	8765	8824	8910
$\bar{X}$	598.0	717.4	759.4	841.0	1016.7	1252.1	1260.6	1272.9

Source of Variation	Degrees of freedom	Sum of squares	Mean Square	F	P
Total	55	4,434,581			
Error	42	526,519	12,536		
Dogs	6	261,905	43,650	3.48	<.01
Treatments	7	3,646,157	520,879	41.55	< .00

After the analysis of variance, the values were analysed for regression before and after thyroxine treatment. The weighted coefficients shown were used to obtain the various components of the treatment sums of squares.

Totals	Thyroxine Effect	Linear (Control)	Linear (T4)	Quadratic (Control)	Quadratic (T4)	Cubic (Control)	Cubic (T4)
4186	-1	-7	0	+7	0	+3	0
5022	-1	-3	0	-4	0	-8	0
5316	-1	+1	0	-8	0	+6	0
5887	-1	+9	0	+5	0	-1	0
7117	+1	0	-7	0	+7	0	+3
8765	+1	0	-3	0	-4	0	-8
8824	+1	0	+1	0	-8	0	+6
8910	+1	0	+9	0	+5	0	-1
Divisor	56	980	980	1078	1078	770	770
Contrast	13,205	13,931	12,900	-3897	11,283	-1609	-473
Sum Sq.	3,113,786	198,033	169,806	13,957	118,094	3363	29,11
F	248.34	15.79	13.54	1.11	9.42	0.26	2.32
P	< 0.001	< 0.001	< 0.005	N.S.	< 0.005	N.S.	N.S.

The coefficients of regression of the linear components were calculated as shown previously, for the group before and after thyroxine treatment.

$$\text{Before thyroxine } \hat{y} = 1137.2X + 6294.5$$

$$\text{After thyroxine } \hat{y} = 1053X + 11,084.3$$

Thyroxine therefore had a highly significant effect ($F = 248.34$) by producing an elevation of the curve as a whole. In addition to the curve having highly significant linear components, both before and after treatment, an effect producing a highly significant quadratic component became evident on analysis after treatment. Before thyroxine the quadratic component was insignificant. In addition to being elevated, the dose-response curve now had a significant curvature with the convexity upwards. This is shown in Fig.26. The calculated linear regression lines are inserted as a thin solid line, whereas the actual values obtained are inserted in bold line.

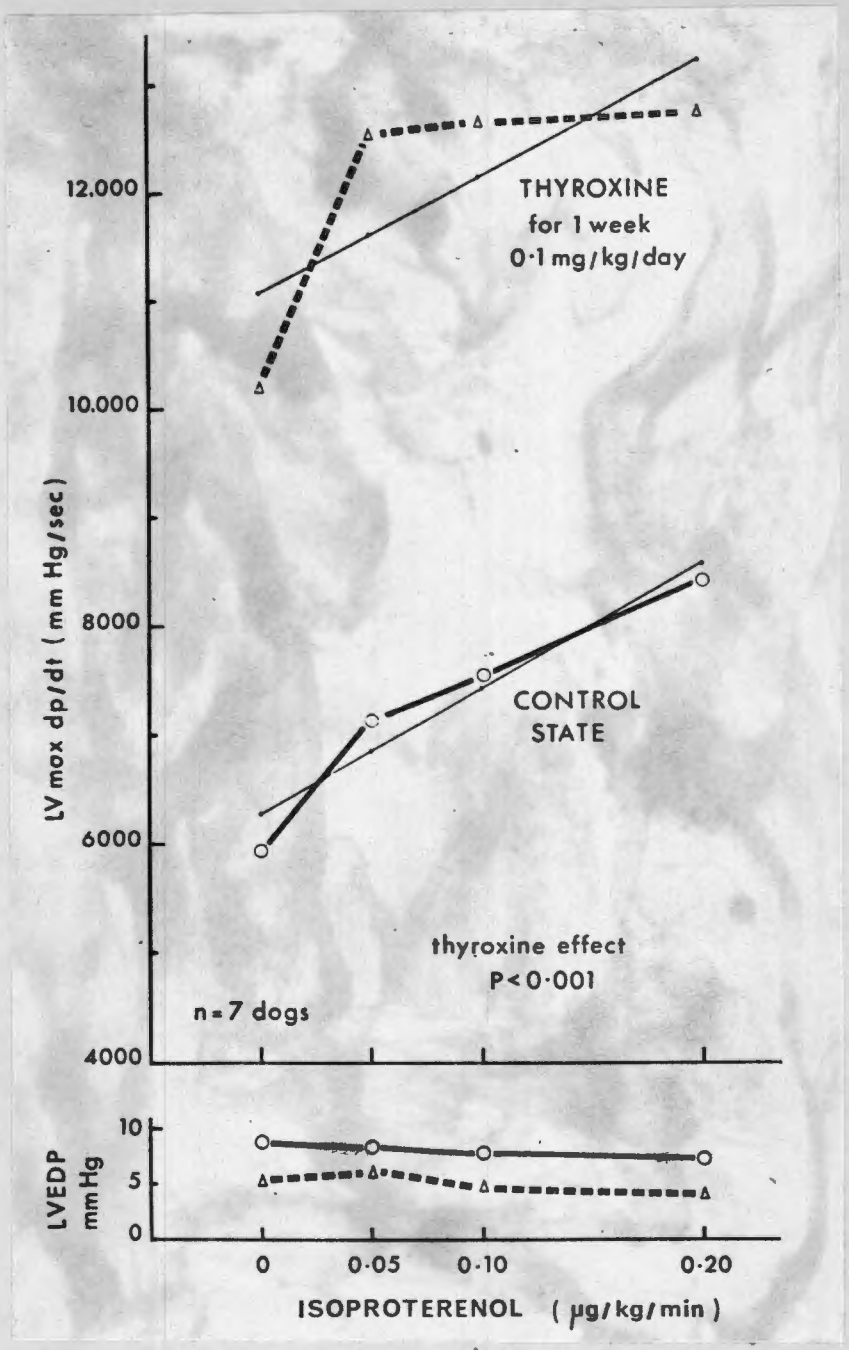


FIG. 26

The data for the other haemodynamic measurements are indicated in summary form in Table XIII. The grand means for the curves (a measure of vertical displacement) are indicated and the F values for the thyroxine effect. Since all curves had linear regression components with no differences in coefficients, these are not indicated. Those instances where a significant quadratic component appeared after thyroxine treatment only are indicated with an asterisk.

TABLE XIII.

	$\bar{Y}$ before thyroxine	$\bar{Y}$ after Thyroxine	F for Thyroxine effect	P
LVmax dp/dt (mm Hg/sec)	7,289	12,006*	248.34	<.001
Heart rate (per min)	92.8	132.8	131.20	<.001
Cardiac output (ml/min)	2,724	3,566	42.68	<.001
Stroke volume (ml)	29.40	27.04	5.81	<.025
Systolic pressure (mm Hg)	157.8	170.9*	24.60	<.001
Diastolic pressure (mm Hg)	85.0	95.8	27.97	<.001
LVEDP (mm Hg)	8.1	4.8	14.57	<.005
Ejection time (msec)	19.18	15.86	108.0	<.005
Stroke work (gm.meters)	40.1	42.0	1.52	N.S.
Stroke power (gm/sec)	210.8	268.2*	44.74	<.001
Syst. ejection rate (ml/sec)	190.8	171.5	12.42	<.005
Periph. resistance (dyn.sec/cm^5)	3359	2883	11.40	<.005

In normal dogs, an infusion of isoproterenol in the doses 0.05, 0.10 and 0.20 µg/kg/min caused an increase in LVmax dp/dt, heart rate and cardiac output with dose-response curves having highly significant linear regression. The effect of treatment with I^{131} was to cause a significant lowering of the entire curve with no alteration in its slope. With the development of frank myxoedema, the curve was lowered to a further significant degree, but the slope was still not significantly different from

that occurring in the normal animals. The effect of thyroxine administration on the other hand, was to cause a significant elevation in the curve, without alteration again in the linear slope, but with a highly significant convexity developing; the initial portion of the curve had a steeper gradient than in the normal state, but as is evident in Fig.25, failure to maintain the linear slope occurs at the higher doses employed. Thyroxine treatment similarly caused elevation of the dose-response curves for heart rate, cardiac output, stroke volume, systolic and diastolic pressures, ejection time, stroke power and systolic ejection rate. This occurred at an end-diastolic pressure that was significantly lower ($P < .005$), hence a true further increase in contractility is reflected during isoproterenol stimulation, attributable to the prior administration of thyroxine. Of particular significance is the fact that such an effect could not be shown for the curve of left ventricular stroke work but was present to a highly significant degree for stroke power and systolic ejection rate; the factor of rate of action enters into both the latter but not the former measurements.

The interpretation of these findings is complicated by unavoidable factors. Although a reduction in height of dose-response curves could be shown after I^{131} treatment, the slope remained unaltered. This may be attributable to the fact that all the dogs gained significant amounts of weight following I^{131} treatment and since the isoproterenol doses were calculated on a weight basis, these animals all received greater absolute amounts of isoproterenol during the studies in the hypothyroid state compared to their study in the euthyroid state. The relative contribution of this could not be assessed.

Evidence for this was, however, obtained from four serial studies in one severely myxoedematous animal. The animal was studied twice during his severely myxoedematous state and on two occasions after thyroid replacement. The results are depicted graphically in Fig.27.

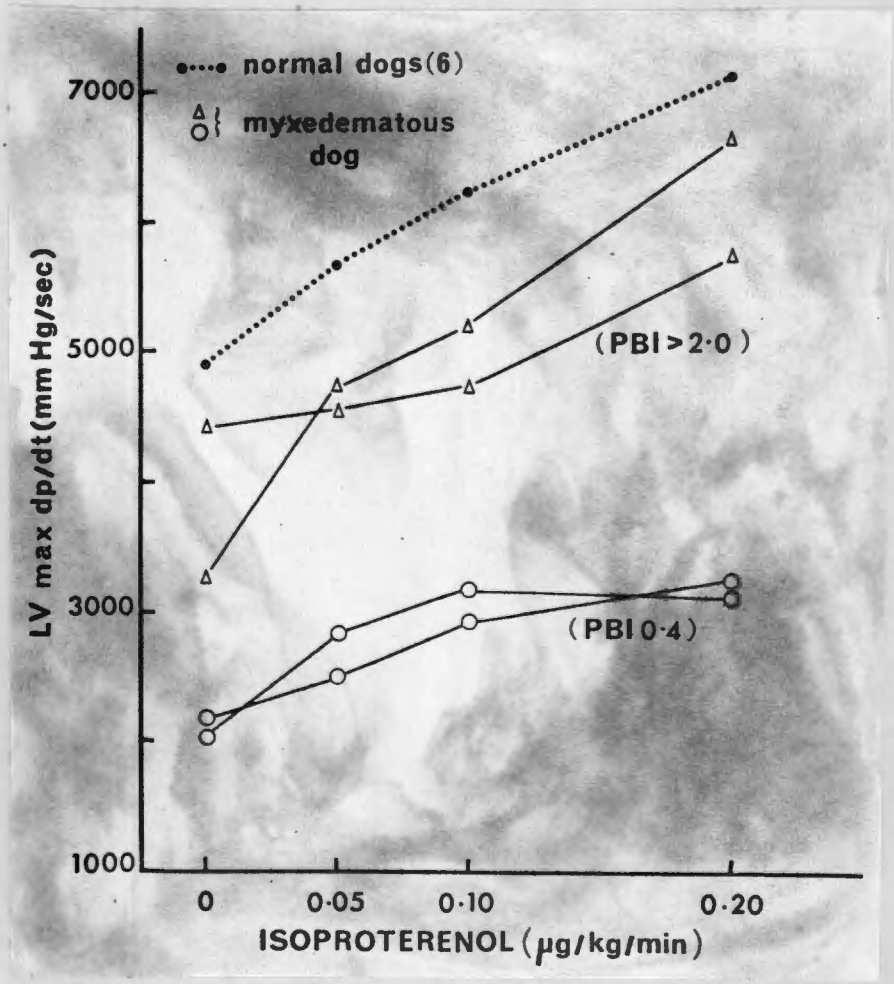


FIG. 27

In this figure is also included the dose-response curve obtained in six normal animals for comparison (the broken line). It is evident that during the two studies performed while in frank myxoedema (PBI = 0.4 µg/ml) the curves were not only depressed but also had a flatter slope than those obtained after administration of thyroid powder (U.S.P.), 60 mg daily, for one month.

The dog weighed 2.4 kg less after thyroid replacement (studies 2 weeks apart) and therefore received a smaller absolute amount of isoproterenol; yet the slope of the curves appeared to have been increased. Statistical tests were not performed since only the one study was possible.

In the experiments involving treatment with thyroxine it became evident that extreme hypersensitivity to isoproterenol may have arisen and that the curves obtained may already be situated in the plateau. Six further experiments were therefore performed inserting dose levels of 0.01 and 0.03 µg/kg/min before the usual lowest dose of 0.05 µg/kg/min. Similar phenomena were obtained; three of the typical experiments are shown in Fig.28.

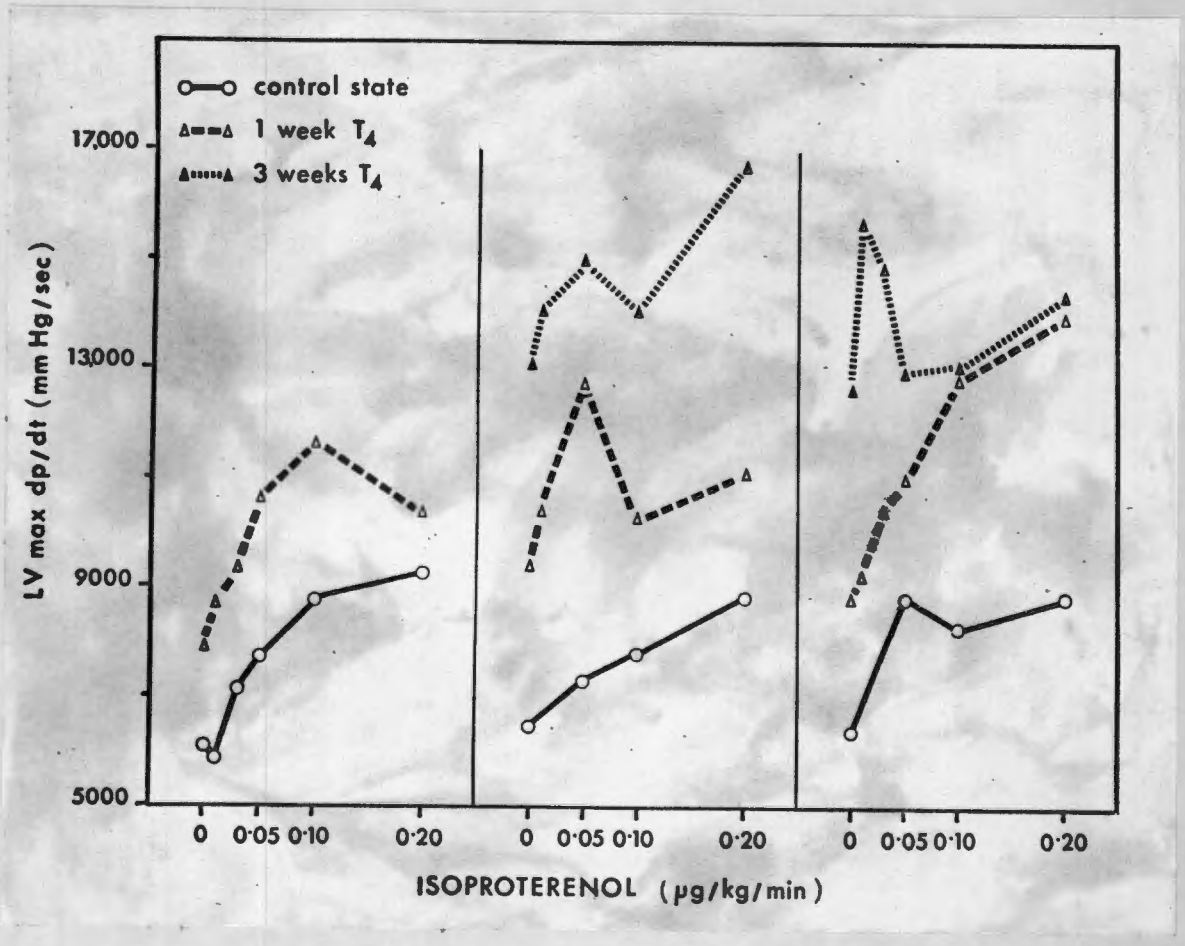


FIG. 28

In the left hand panel it is evident that the dog responded with an elevation of 800 mm Hg to the lowest dose used (0.01 µg/kg/min) after one week of treatment with thyroxine, whereas no response was obtained during the control study for this dose. The curve then continued with the characteristic curvature and actually faltered at the highest dose level with a smaller response than at the 0.10 µg/kg/min level.

In the centre panel, the characteristic elevation of the curve with the marked hyperresponsiveness at the lower dose levels is demonstrated. In the right hand panel there was no hyperresponsiveness to the low levels at one week of treatment, but this phenomenon was markedly present associated with massive elevation of the curve after three weeks of treatment with thyroxine.

CONCLUSIONS:

The responses of the myocardium to beta-adrenergic stimulation are influenced in opposing directions by induction of hypothyroid and hyperthyroid states. Large changes in the elevation of the dose-response curves to isoproterenol could be demonstrated, but the linear slopes remained unaffected. In the hypothyroid state, the changes in slope may have been prevented by the administration of larger amounts of isoproterenol calculated on the basis of the body weight of the animals, all of which had gained weight. In the hyperthyroid state the linear dose-response relationships are completely different; the heart has increased responsiveness at very low dose levels, but cannot maintain the same dose-response relationships, with the development of a marked convexity in the dose-response curve; these

changes occur at a greatly elevated level of contractility compared to the control state.

ADDENDUM:

Since the development of the curvature in the dose-response curve occurred at very high levels of function, the question arose whether this may be a failure of the frequency-response characteristics of the recording system, rather than of the myocardium or its control mechanisms.

This was evaluated by repeating the experiment in one animal; the curve with its usual convexity was found to reach a plateau at 14,000 mm Hg/sec. Atropine was then injected (0.1 mg/kg intravenously) and the isoproterenol infusion repeated. The initial portion of the curve remained unaltered, but at the 0.10 and 0.20 µg/kg/min dose levels of isoproterenol, levels of 17,800 and 19,000 mm Hg/sec were obtained. The values obtained in this dog are supplied in Appendix Table 4.

It became clear therefore that the apparatus was quite capable of performing at levels well beyond the apices of these curves; the response to atropine suggested that the vagus nerve must have a powerful inhibitory influence at such levels and may be the factor, rather than a myocardial one, responsible for the convexity in the dose-response occurring at such high levels of performance.

4. RELATIVE CONTRIBUTIONS OF SENSITIZATION TO ENDOGENOUS CATECHOLAMINES AND DIRECT EFFECT OF THE HORMONES ON THE MYOCARDIUM.

Since it was shown that an alteration in responsiveness to beta-adrenergic stimulation could be induced by the induction

of an alteration in thyroid state, it was thought relevant to assess the relative influences of altered sensitivity to catecholamines and the direct effect of the hormone on the muscle in the production of the altered resting levels of myocardial contractility.

The beta-adrenergic blocking agent, propranolol, was utilized in the group of animals treated with l-thyroxine. The response to propranolol and final level of LVmax dp/dt obtained after complete blockade, (judged by failure to respond with an increase of rate or LVmax dp/dt to an isoproterenol infusion of 0.50 μg/kg/min), were obtained during the study in the euthyroid state and again in the hyperthyroid state accomplished by daily injections of l-thyroxine, 0.1 mg/kg, for one week. It has already been demonstrated that the haemodynamic changes of hyperthyroidism can be induced in this period of thyroxine treatment. Readings were obtained 20 minutes after the administration of propranolol intravenously, 0.4 mg/kg. The results are summarized in Fig.29. This shows the resting levels of LVmax dp/dt in both the euthyroid and hyperthyroid state before and after blockade with propranolol. Details are supplied in Appendix Table 5.

Statistical evaluation of the changes induced by propranolol, utilizing the "t-test" for paired values, revealed a significant increase in the response to propranolol in the hyperthyroid state. The mean response obtained in the euthyroid state was 1922 mm Hg/sec reduction in LVmax dp/dt; in the hyperthyroid state the mean response was 3640 mm Hg/sec. This value is significantly greater than the value obtained in the euthyroid state ($P < 0.05$).

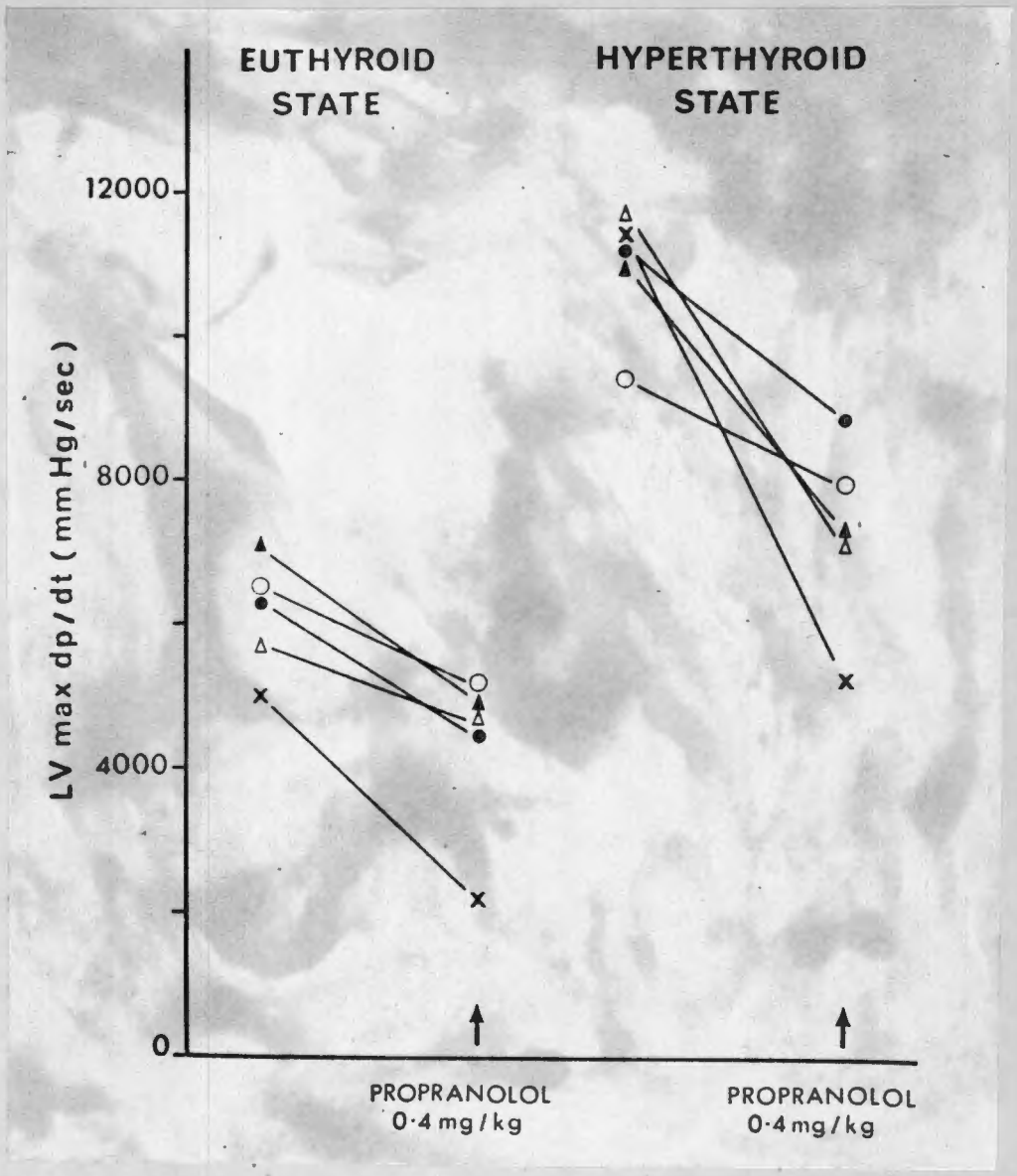


FIG.29

The mean resting value for LVmax dp/dt was 6122 mm Hg/sec before propranolol in the euthyroid state and 10,884 mm Hg/sec in the hyperthyroid state. These values were highly significantly different ($P<0.001$). The mean resting value after propranolol was 4200 mm Hg/sec in the euthyroid state and 7244 mm Hg/sec in the hyperthyroid state. These values were also highly significantly different ($P<0.005$). In none of the dogs did the value of LVmax dp/dt after propranolol blockade in the hyperthyroid state approach the levels of LVmax dp/dt even before propranolol in the euthyroid state. It is therefore clear that

after complete blockade of the beta-adrenergic receptors, a highly significant effect remains following treatment with thyroxine.

The effects on heart rate were not as easy to evaluate. The mean effect of propranolol on the five animals in the euthyroid state was 14 beats/min reduction; in the hyperthyroid state the mean effect was 18 beats/min reduction. These values approached, but did not achieve statistical significance ($P < 0.20$). The statistical evaluation would have been more valid if larger numbers could have been studied. The mean rate before propranolol in the euthyroid state was 78 beats/min and 110 beats/min in the hyperthyroid state; after propranolol the rates were 64 in the euthyroid state and 92 in the hyperthyroid state.

Data on cardiac output were not analysed because of the poor quality dye-dilution curves obtained after propranolol in the euthyroid state; this was as a consequence of both the slow rate and the reduction in contractility after propranolol.

CONCLUSIONS:

By means of blockade of the beta-adrenergic receptors with propranolol it has been possible to demonstrate that the increased myocardial contractility encountered in the hyperthyroid animal was composed of a greater than normal fraction due to stimulation by endogenous beta-adrenergic factors and also a component independent of the beta-adrenergic receptors and attributable to a direct effect of the hormone.

ADDENDUM:

As well as evaluating the role played by the adrenergic system in the manifestation of the hyperthyroid state in the dogs, it was thought relevant to attempt an assessment of the possible role of the parasympathetic system in the maintenance of the low heart rate and low contractility of the myxoedematous animal. Only one experiment was possible (the other three animals died, one from an acute myocardial infarction in the presence of patent coronary arteries, sustained during the infusion of isoproterenol.

This myxoedematous dog had a resting heart rate of 38 beats per minute and a resting level of LVmax dp/dt of 2210 mm Hg/sec. The dog was then given atropine 0.1 mg/kg; the heart rate increased to 150 beats per minute, representing a 300% increase whereas LVmax dp/dt increased to a steady-state peak level of 3690 mm Hg/sec, representing a 66% increase. Although one could not extrapolate about the role of the parasympathetic system in the manifestation of the cardiac state of myxoedema, one could draw the conclusion that the heart rate is controlled to a larger extent than myocardial contractility by the parasympathetic system.

5. EFFECT OF L-TRIIODOTHYRONINE ON RESTING HAEMODYNAMICS AND RESPONSES TO ISOPROTERENOL.

It was shown that the treatment with daily injections of l-thyroxine induced an elevation in the resting levels of LVmax dp/dt and an upward displacement of the dose-response curve to isoproterenol. Since triiodothyronine (T3) has been shown to be a rapidly acting form of thyroid hormone (113) assessment of its effects on the responses of the dog heart to

beta-adrenergic stimulation was thought to be pertinent.

16 normal and 6 hypothyroid dogs were used in these experiments. Infusions of isoproterenol in the doses 0.05, 0.10 and 0.20 µg/kg/min were used, each dose being infused for 12 minutes. Following the completion of infusion of the highest dose used, the dog was allowed 90 minutes to recover and repeat baseline observations were obtained of pressures, heart rate and cardiac output. An infusion of l-triiodothyronine in the dose 0.1 µg/kg/min was then given for 15 minutes and repeat observations obtained. The isoproterenol infusions were then repeated in the same doses for the same periods. In an additional 6 control experiments the procedure was the same, except that the triiodothyronine was not added to the 0.1 N sodium hydroxide solution and the infusion of solvent diluted in physiological saline was given by itself. In an additional four experiments, the dextro-isomer of triiodothyronine was used in order to establish whether the effects observed were specific for l-triiodothyronine. The solvent and d-T3 control experiments were performed in normal dogs only.

The detailed values are in the Appendix Table 6. The statistical method used was a two-way analysis of variance with partitioning of the treatment sum of squares into displacement and regression (linear, quadratic and cubic) components. The analyses of variance for LVmax dp/dt and heart rate in the euthyroid experimental and control groups are given in Table XIV while the alterations in grand means of the response curves are shown for the other haemodynamic measurements, in all the groups, in Table XV.

TABLE XIV.

EXPERIMENTAL GROUP (EUTHYROID).

LVmax dp/dt (coded X 10^{-1}).

Source of variation	Degrees of Freedom	Sum of Squares	F	P
Total	53	901,993		
Error	40	322,921		
Dogs	5	117,674	2.92	<.025
Treatments	8	461,398	7.14	<.005
(a) T3 effect on resting values.	1	936	0.11	N.S.
(b) T3 effect on isoproterenol responses	1	61,061	7.56	<.01
(c) Pooled linear regression	1	326,820	40.48	<.001
(d) Pooled quadratic regression	1	10,010	1.24	N.S.
(e) Pooled cubic regression	1	169	0.02	N.S.
Heart rate:				
Total	53	19,965		
Error	40	4,124		
Dogs	5	2,060	3.9	<.01
Treatments	8	13,781	16.7	<.001
(a) T3 effect on resting values	1	21	0.2	N.S.
(b) T3 effect on isoproterenol responses	1	1	0	N.S.
(c) Pooled linear regression	1	11,639	112.8	<.001
(d) Pooled quadratic regression	1	78	0.75	N.S.
(e) Pooled cubic regression	1	11	0.10	N.S.

CONTROL GROUP (SOLVENT INFUSION).

LVmax dp/dt (coded X 10^{-1}).

Source of variation	Degrees of Freedom	Sum of Squares	F	P
Total	53	1,119,034		
Error	40	147,443		
Dogs	5	669,112	36.30	< .001
Treatments	8	302,479	10.25	< .001
(a) Solvent effect on resting values	1	2,916	0.79	N.S.
(b) Solvent effect on iso-proterenol responses	1	37	0.00	N.S.
(c) Pooled linear regression	1	248,979	67.54	< .001
(d) Pooled quadratic regression	1	9,190	2.49	N.S.
(e) Pooled cubic regression	1	1,314	0.35	N.S.
Heart rate:				
Total	53	12,728		
Error	40	3,746		
Dogs	5	2,435	5.20	< .005
Treatments	8	6,547	8.73	< .005
(a) Solvent effect on resting values	1	85	0.91	N.S.
(b) Solvent effect on iso-proterenol responses	1	(-)462	4.93	N.S.
(c) Pooled linear regression	1	5,490	58.62	< .001
(d) Pooled quadratic regression	1	27	0.28	< .001
(e) Pooled cubic regression	1	14	0.15	N.S.

The regression components are presented by pooling the values before and with T3 since no differences in the coefficients of regression were obtained on analysis of these before and after

1-T3 administration.

The alterations in grand means (a measurement of vertical displacement) are presented for all the haemodynamic measurements in Table XV.

TABLE XV.

1. Euthyroid Experimental Group:

	$\bar{Y}$ before T3	$\bar{Y}$ after T3	F.	P.
LVmax dp/dt (mm Hg/sec)	5954	6667	7.56	<.01
Heart rate (per min)	88	88	0	N.S.
Cardiac output (ml/min)	2499	2472	0.02	N.S.
Stroke volume (ml)	27.5	26.8	0.19	N.S.
MAP (mm Hg)	110	114	3.54	N.S.
LV stroke work (gm meters)	37.6	37.5	0	N.S.
LV stroke power (gm meters/sec)	190	218	10.11	<.005
Syst. eject. rate (ml/sec	139	155	7.24	<.02
Ejection time (msec)	197	173	56.06	<.001
LVEDP (mm Hg)	9.4	8.5	1.32	N.S.
Periph. resistance (dyn.sec/cm^5)	4334	4874	2.94	N.S.

2. Solvent Control Group:

	$\bar{Y}$ before T3	$\bar{Y}$ after T3	F.	P.
LVmax dp/dt (mm Hg/sec)	6271	6288	0	N.S.
Heart rate (per min)	85	78	4.93	<.05
Cardiac output (ml/min)	2654	2148	6.63	<.025
Stroke volume (ml)	31.8	27.5	9.21	<.01
MAP (mm Hg)	112	120	12.76	<.005
LV stroke work (gm meters)	44.2	40.9	3.02	N.S.
LV stroke power (gm meters/sec)	231	227	0.08	N.S.
Syst. eject. rate (ml/sec)	165	154	1.69	N.S.
Ejection time (msec)	194	179	43.8	<.001
LVEDP (mm Hg)	7.8	9.0	6.89	<.025
Periph. resistance (dyn.sec/cm^5)	3706	5526	24.02	<.001

3. Hypothyroid Group:

	$\bar{Y}$ before T3	$\bar{Y}$ after T3	F.	P.
LVmax dp/dt (mm Hg/sec)	4575	5437	36.92	$<.001$
Heart rate (per min)	79	76	4.18	$<.05$
Cardiac output (ml/min)	2140	2215	0.95	N.S.
Stroke volume (ml)	26.9	28.8	4.39	$<.05$
Mean arterial pressure (mm Hg)	101.8	110.3	13.65	$<.005$
LV stroke work (gm meters)	33.2	40.3	26.21	$<.001$
LV stroke power (gm meters/sec)	159.6	199.6	37.04	$<.001$
Systolic ejection rate (ml/sec)	129.3	143.0	9.71	$<.005$
Ejection time (msec)	207	201	4.18	$<.05$
LVEDP (mm Hg)	8.98	8.62	0.52	N.S.
Peripheral resistance (dyn.sec/cm^5)	4307	4643	1.65	N.S.
4. d-T3 Control Group:				
LVmax dp/dt (mm Hg/sec)	7531	7467	0.11	N.S.
Heart rate (per min)	92	100	4.35	$<.05$
Cardiac output (ml/min)	2751	2428	3.61	N.S.
Mean arterial pressure (mm Hg)	104	114	23.08	$<.001$
Stroke power (gm meters/sec)	211	209	0.04	N.S.
Systolic ejection rate (ml/sec)	156	142	4.37	$<.05$
LVEDP (mm Hg)	6.06	6.88	1.53	N.S.

When the isoproterenol infusions were administered in the presence of an l-triiodothyronine infusion, the dose-response curves were significantly higher for LVmax dp/dt, left ventricular stroke power and systolic ejection rate. This was accomlished without any alteration in the baseline levels, or in the heart rate responses. These ventricles were therefore responding with more rapid generation of pressure and with more rapid shortening in the absence of an increase in heart rate or in cardiac output. The velocity-dependence of the effect is emphasized by the absence of any effect on responses of either stroke volume or stroke work, but the presence of highly significant increases in stroke power and systolic ejection rate. The effect was specific for the presence of l-triiodothyronine (0.1 μg/kg/min) in the infusate and could not be shown either during infusions of the solvent alone or with infusions of d-triiodothyronine in the same dose. The curves for LVmax dp/dt are shown in Fig.30.

The responses obtained in the euthyroid group are also apparent in the hypothyroid group, except that they were obtained with higher degrees of significance. The hypothyroid animals, too, in contradistinction to the euthyroid group, had a significant reduction in the responses of heart rate. This is particularly notable in view of the higher levels of significance of the velocity-dependent measurements. The hypothyroid animals in contradistinction to the normal group also had increased responses of mean arterial pressure ($P < 0.005$). This caused the emergence of significantly increased responses of left ventricular stroke work in this group.

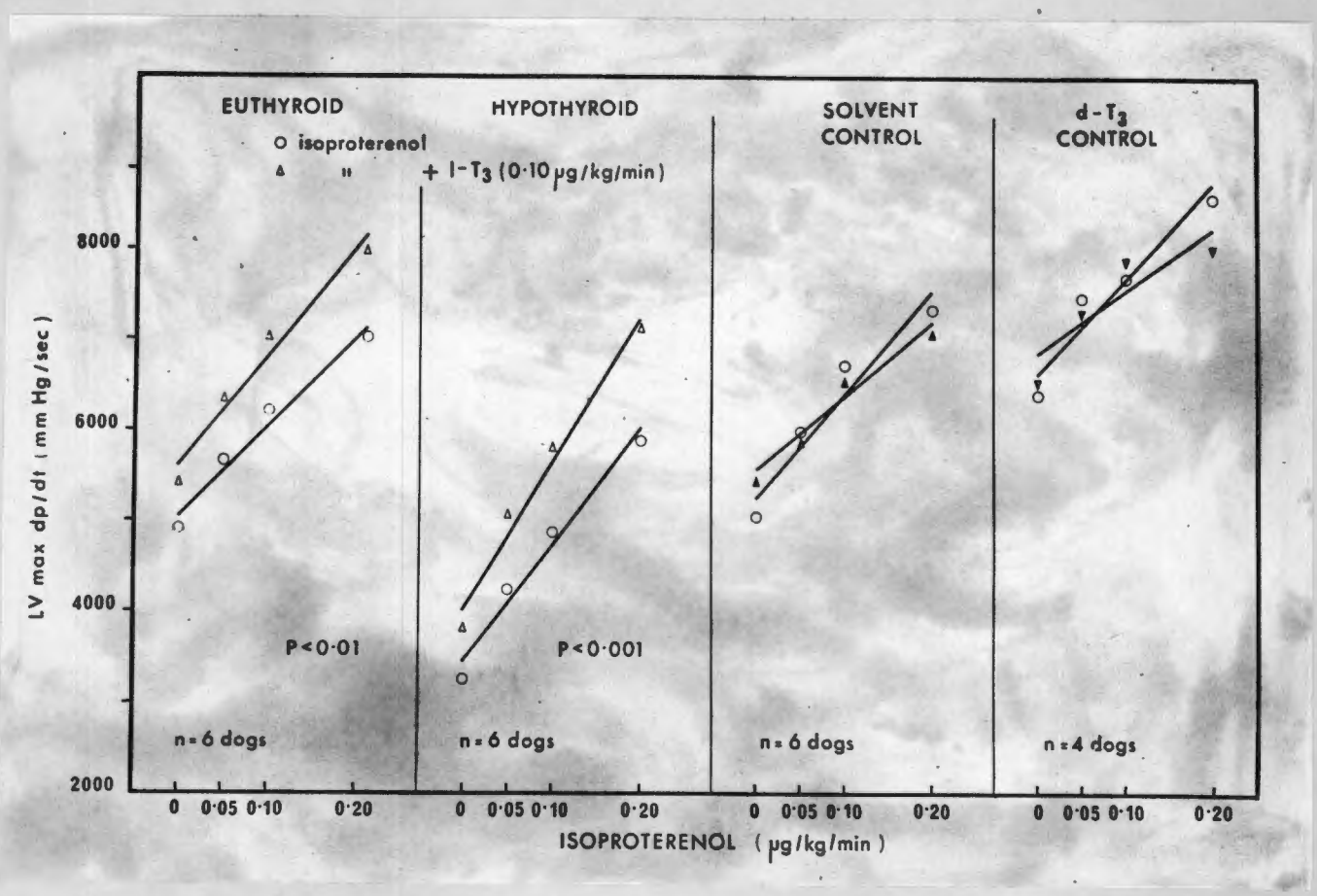


FIG.30

In the control group a similar result was obtained in that the mean arterial pressure was higher during the infusions of isoproterenol and solvent. LVEDP was also, however, increased significantly ($P < .025$). These factors may account for the significant reduction in ejection time observed in this group and is reflected by the highly significant increase in calculated peripheral resistance. The change in mean arterial pressure responses was also present in the group of animals receiving d-T3 instead of l-T3.

These effects were obtained after infusions of l-T3 lasting 15 minutes. In order to define the time required for the effect to develop experiments were performed in four animals in which

l-T3 was administered during an infusion of isoproterenol, while a continuous slow-speed recording of LVmax dp/dt was obtained. In two dogs the l-T3 was given by continuous infusion at the rate of 0.1 µg/kg/min. In the other two animals the total dose for 15 minutes was given as a single injection. Such an experiment is illustrated in Fig.31.

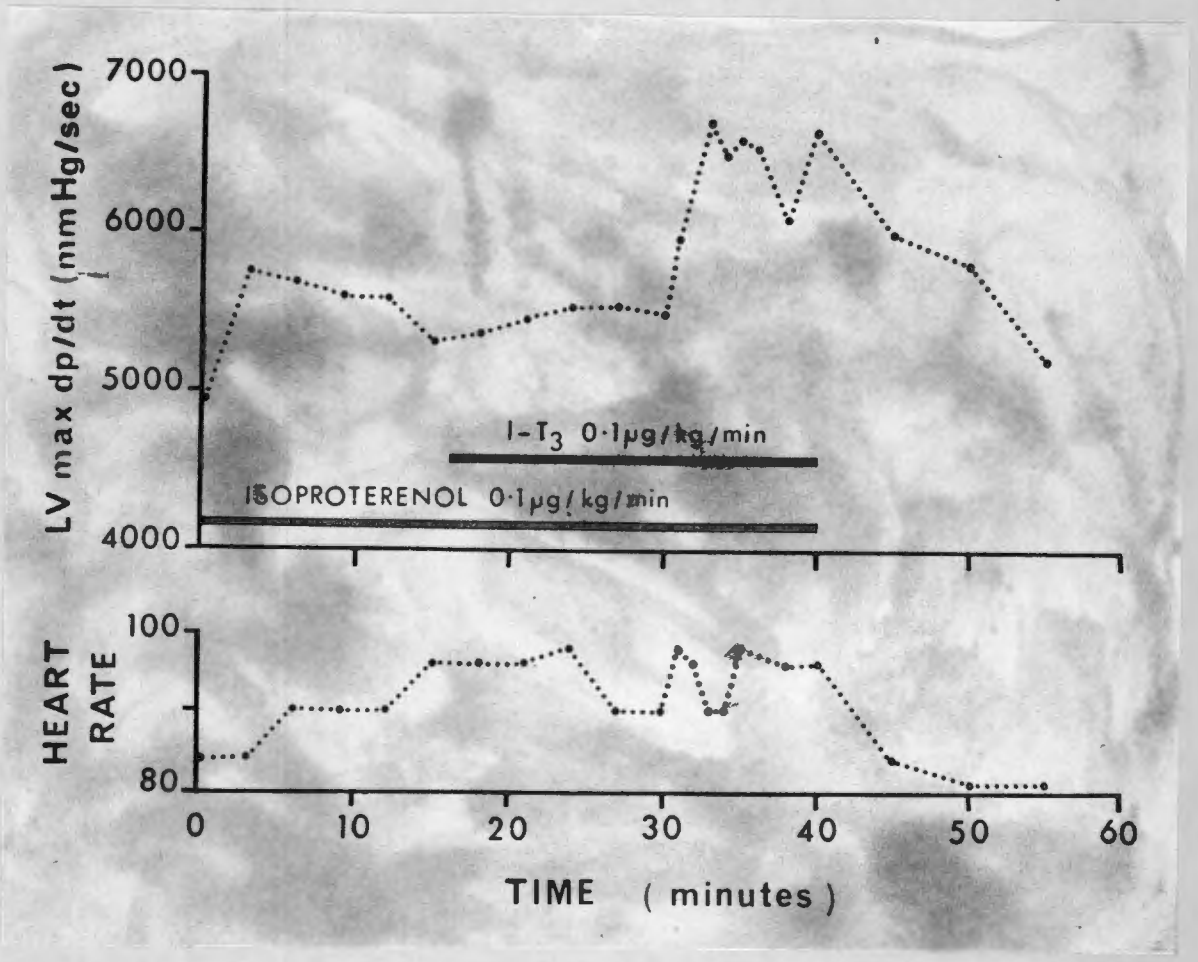


FIG.31

This reveals an increase in LVmax dp/dt commencing 15 minutes after the start of the l-T3 infusion. No increase in heart rate was shown to occur at the same time. The times obtained for the onset of action of l-T3 in the other 3 dogs were 8 minutes, 11 minutes and 12 minutes. It would appear to be mediated therefore in a matter of approximately 10 minutes.

Four control experiments were performed in which only the

solvent of the l-T3 was injected (2 dogs) or infused (2 dogs) during the isoproterenol infusion. No change in LVmax dp/dt was seen in any of these animals. This proved that the effect was caused by the presence of l-triiodothyronine.

CONCLUSIONS:

l-Triiodothyronine can be shown to have an effect on the responses of the myocardium to a beta-adrenergic stress within 15 minutes of its administration. The effect is inotropic without any chronotropic manifestations in the periods studied and yet becomes apparent on the velocity-dependent aspects of ventricular function. The effect was not apparent on the resting ventricle in the time of observation in these experiments. An additional increase in myocardial contractility over and above that caused by the isoproterenol became apparent and is ascribable to the presence of l-triiodothyronine.

DISCUSSION.

1. LVmax dp/dt as a Measure of Myocardial Contractility:

In these experiments LVmax dp/dt was found to be a useful index of the contractile state of the myocardium. It was found not only to be a sensitive measure, but was altered under all circumstances in parallel fashion with other measurements of myocardial contractility; ventricular stroke work, power and rate of ejection. In these respects our results correlate well with the findings of Knopp et al,[90] Murphy et al [111] and Covell et al.[76] Our findings support particularly the views of the latter group of authors who also found this measurement to be a more sensitive index of myocardial contractility than those based on the Frank-Starling principle. In their comparative study LVmax dp/dt was second only to direct measurement of the force-velocity relations in isolated isovolumic beats obtained with much more sophisticated apparatus and previous surgical intervention. LVmax dp/dt can be obtained continuously, involves relatively simple electronic apparatus and its measurement through a catheter-manometer system appears sufficiently accurate if precautions against damping of the system are taken. A valuable insight into directional changes is at least obtained with such a system. In our experiments excellent correlation was obtained with an intracardiac micromanometer.

The importance of measuring the rate of pressure, and indirectly rate of tension development rather than total tension developed becomes obvious, even though the latter measurement may be used with benefit under certain circumstances, in the isolated myocardial preparation using the Walton-Brodie strain

gauge and allowing the afterload to change.[8,114] For such changes to be interpreted as alterations in intrinsic contractile state, end-diastolic fibre length must not have increased in length.

Substantial alterations in myocardial contractility may be overlooked if height of peripheral arterial pressure rather than the rate of ventricular pressure development is measured. In these experiments, mean aortic pressure was found not to be different from normal in animals recently treated with I^{131} despite significant changes in body weight, serum cholesterol, heart rate and LVmax dp/dt. It was only with the development of frank myxoedema that the mean arterial pressure became significantly reduced. In the animals treated with thyroxine the mean arterial pressure was initially increased but after two further weeks of treatment this measurement was no different from the normal level, in contradistinction to LVmax dp/dt which was grossly elevated. LVmax dp/dt is therefore a more direct measure of events at a myocardial level than is peripheral pressure which is profoundly influenced by the state of peripheral arterial tone.

In their in-vitro preparation, Buccino et al[115] succeeded in demonstrating, in cat papillary muscles, profound effects of thyroid hormone on the <u>rate</u> of tension development without alteration in the total tension developed. The administration of l-triiodothyronine for 15 minutes in our experiments augmented exclusively the velocity-dependent aspects of myocardial action at a time when no alteration in pressure or volume-dependent measurements could be demonstrated. The increases in LVmax

dp/dt (Table XV) occurred in parallel with observed shortening of left ventricular ejection period and with increases of the calculated indices, left ventricular stroke power and systolic ejection rate. These effects were observed in the absence of any increase in heart rate. It becomes apparent that, particularly with reference to the action of thyroid hormone, it is important to assess those aspects of myocardial function that incorporate the velocity of contraction.

When the inotropic effects of thyroid hormone were accompanied by chronotropic effects, the percentage changes were much greater for LVmax dp/dt than for heart rate or cardiac output. This is illustrated in Table XVI which represents an analysis of these pooled measurements in the animals treated with l-thyroxine.

TABLE XVI.

	HEART RATE (per min)	CARDIAC OUTPUT (ml/min)	LV max dp/dt, mm Hg/sec
CONTROL STATE	79	2286	6110
AFTER THYROXINE (0.1 mg/kg/day) 7 days	110 (↑38.3%)	3028 (↑32.4%)	10,410 (↑70.3%)
21 days	143 (↑81.2%)	2996 (↑31.0%)	11,644 (↑90.6%)

Mean values for the same 5 dogs in all periods.

The percentage increase in LVmax dp/dt was greater at an earlier stage and exceeded the increases in heart rate and cardiac output even with prolongation of treatment.

These considerations may account for the failure of Benforado and Wiggins[116] to demonstrate differences in the performance of ventricular muscle strips from normal and hypothyroid rats. These authors measured total tension developed in animals six weeks after treatment with I^{131} when significant alterations in body weight, heart rate and basal metabolic rate could be demonstrated. Of particular interest is their failure to show differences in the tensions developed during increases in rate of stimulation to 320 contractions per minute. Their findings are in striking contrast to those of Buccino et al.[115]

2. Myocardial Contractility and Thyroid Status in the Intact Dog.

Treatment with I^{131} in these dogs caused a reduction in heart rate, cardiac index, peak systolic pressure and LVmax dp/dt, evident as early as six weeks after treatment. These changes were progressive until, in the frankly myxoedematous dogs, studied more than two years after I^{131} administration, the complete syndrome of myxoedematous heart disease [117,118] had developed. These animals had a lower pulse rate and cardiac index when compared to the normal dogs and dogs treated less than one year previously with I^{131}. All the criteria of myocardial contractility were reduced to a highly significant degree in the left ventricle and in the right ventricle a highly significant reduction of the maximal rate of pressure development was shown. The possibility that these haemodynamic manifestations were

mediated through complications of the hypothyroid process e.g. atherosclerotic narrowing of the coronary arteries [119] or pericardial effusions[120] was ruled out by the demonstration of the absence of both these changes at post-mortem examination, including coronary arteriography. To complete the picture, the animals had gained weight, were slow in their movements and failed to grow hair in areas where it had been shaved. They had lard-like subcutaneous tissues and highly elevated levels of serum cholesterol. One of these animals died four days after an isoproterenol infusion. Autopsy and histological examination revealed an acute myocardial infarct involving the left ventricle, in the presence of patent coronary arteries.

On the other hand, the administration of 0.1 μg/kg l-thyroxine daily by subcutaneous injection, resulted in striking haemodynamic changes, comparable to those of hyperthyroid heart disease, [118,121] as early as one week after the commencement of treatment. These changes, summarized in Table VI(b) showed an increase in LVmax dp/dt, heart rate, cardiac index (without a change in stroke volume index) and a significant reduction in systolic ejection period. LVmax dp/dt and stroke power were significantly increased for the left ventricle and the maximum rate of rise of pressure in the right ventricle was also shown to be highly significantly increased. With further treatment for two weeks, body weight decreased progressively, but significant increases in heart rate and contractility could not be demonstrated. The peripheral resistance index had dropped significantly by the third week of treatment. Our observations of the maintenance of a normal stroke volume and mean systolic

ejection rate is in support of the finding by Goldstein and Killip[122] that both these calculated values remained unchanged in their patients with thyrotoxicosis on catecholamine depletion with guanethidine, in contradistinction to the other haemodynamic measurements which became reduced.

These dogs were generally excitable, invariably developed diarrhoea but were adequately sedated by the same dose of morphine administered during their euthyroid state. Ventricular fibrillation occurred in two of these animals during isoproterenol infusions and a third died from irreversible acute left ventricular failure that occurred during an isoproterenol infusion. The LVmax dp/dt of this animal had reached the level of 16,000 mm Hg/sec. The occurrence of cardiac failure in previously normal dogs as a result of the administration of exogenous thyroid has been documented by Piatnek-Leunissen and Olson.[123]

The changes in myocardial contractility caused by the induction of hypothyroidism and hyperthyroidism were diametrically opposite and progressive. Myocardial contractility can therefore in the intact animal be correlated with the thyroid status of the animal. This is in support of the findings of Buccino et al in papillary muscles from such animals.[115] The findings of these authors have indicated that these changes in myocardial contractility are of a primary nature and manifest a direct effect of the hormone on the functional characteristics of the contractile mechanism. Views have previously been expressed that the haemodynamic manifestations of thyroid heart disease in the intact subject are entirely secondary to peripheral

actions of the hormone. Resnik and Harrison[118] and Howitt and Rowlands[121] suggested that the alterations in cardiovascular function were secondary compensatory manifestations of the changes in peripheral metabolic requirements. Theilen and Wilson[124] invoked alterations in peripheral vasoconstriction, on the basis of excessive response of atropinized thyrotoxic individuals to phenylephrine, as being the primary source of the haemodynamic alterations, whereas Harrison[125] supported the view that alteration of function in the adrenergic system induced by the abnormal thyroid state was the primary pathophysiologic mediator. Aumann and Youmans[126] attempted to invoke differential sensitization of adrenergic neuroeffector systems as being primarily involved with the cardiovascular manifestations secondary to these. The demonstration by Buccino et al[115] that profound alterations in contractile state of myocardial muscle, devoid of any nervous or metabolic influences can be induced by thyroxine administration, have resolved most of these arguments and point to a definite myocardial effect of the hormone.

Our experiments have succeeded in demonstrating, by direct measurement, the alterations induced by a change of thyroid status in the intact animal. Several authors have attempted to demonstrate this by indirect assessment of the functional state of the heart. De Groot et al[127] attempted by simultaneous recording of the carotid pulse and the phonocardiogram to demonstrate a reduction in the isometric contraction period and systolic ejection time in hyperthyroidism. These changes could, however, be mere manifestations of the tachycardia

encountered in this state. Howitt and Rowlands[128] have likewise attempted by the measurement of the systolic ejection rate to quantitate the alterations in myocardial contractile state in thyrotoxic patients. Our findings and those of Goldstein and Killip[122] demonstrate that these calculated derivatives may remain normal in the presence of marked alterations in contractility, since both numerator (stroke volume) and denominator (ejection time) are reduced as a result of the accompanying tachycardia. Howitt et al[129] have recently used the rate of rise of pressure in the aorta as an index of myocardial function. This measurement is determined not only by the velocity of ejection of blood from the left ventricle, but also by the distensibility of the systemic arterial bed and by the mass of blood that is accelerated.[130] It can therefore at best be used as a mere reflection of ventricular function. These authors nevertheless came to the conclusion that myocardial contractility is increased in clinical hyperthyroidism, but could not establish whether the changes in aortic dp/dt they documented were merely mediated through the increase in heart rate. Recently Amidi et al[131] by simultaneous recording of the electrocardiogram, carotid arterial pulse and phonocardiogram, measured left ventricular ejection time and external isovolumic contraction time in normal, hyperthyroid and hypothyroid subjects. From simultaneous determinations of cardiac index and heart rate, predicted values for these measurements were obtained. It was found that in the normal subjects the actual and predicted values were identical, but in the hyperthyroid subjects the actual values were less than the predicted and in hypothyroid subjects exceeded

the predicted values at any particular heart rate. These differences were interpreted as being mediated by primary alterations in myocardial contractile state in these conditions. Ueda et al[132], by transseptal left heart catheterization in 6 cases of hyperthyroid heart disease, demonstrated increased systolic ejection rate and calculated circumferential fibre shortening velocity in the left ventricle. Values for LVmax dp/dt are however not supplied. Their opinion was that the thyrotoxic heart was overloaded by increased venous return and speeded up by thyroid hormone without involvement of a change in contractility.

3. <u>The nature of the increased contractility in hyperthyroid dogs.</u>

(a) <u>Characterization by rate-contractility curves</u>.

As indicated, several groups of investigators have recently speculated that an increased state of myocardial contractility exists in thyrotoxicosis and have attempted to delineate it by various indirect measurements. Although these groups have succeeded in demonstrating that several of these indirect indices of myocardial contractility are increased in thyrotoxicosis and reduced in hypothyroidism[129,131,132] they could, however, not ascertain whether the changes they observed were not merely associated manifestations of the altered heart rates encountered in these states.

Our experiments performed by increasing the heart rate artificially in normal and hyperthyroid dogs have indicated that although a fraction of the increased myocardial contractility was due to the autoregulatory increase associated with the increased heart rate, at all rates the measurements obtained of

LVmax dp/dt in the hyperthyroid state were in excess of the expected level attributable to an increase in heart rate. The rate-LVmax dp/dt curves furthermore indicated that although the autoregulatory mechanism is reset to operate at a higher level of contractility, reflected by the vertical displacement of the curve, there was also an alteration in the rate-contractility mechanism, reflected by the increased coefficients of regression shown in four of the five animals. This, however, appeared to account for a relatively insignificant fraction of the entire increase in contractilfty. A control experiment performed indicated the specificity of the displacement of the curves for the presence of l-T4 in the daily injections. Care was also taken in these experiments to utilize the normal conduction pathways by confining observations to atrial pacing without evidence of heart block. This accounts for the fact that these experiments could not be performed in the hypothyroid animals, since these animals manifested varying degrees of A-V block, even at rates not substantially greater than their resting rates.

The use of morphine as a sedative may have contributed to these prohibitive difficulties encountered in the hypothyroid dogs since this agent is known to increase vagal effects in the intact animal[133,134] and it has recently been postulated that the parasympathetic system may have increased tone in the hypothyroid state.[135] On the other hand it was a particularly suitable agent to use since it has been demonstrated not to have any direct inhibitory effect on myocardial contractility,[136] particularly as assessed by rate-LVmax dp/dt curves.[137]

Our observations answer directly the questions put by Howitt et al[129] who felt that although they could not demon-

strate the primary increase in myocardial contractility, that it had to be there and was probably over and above the rate-related increment.

(b) Characterization by beta-adrenergic blockade with propranolol

The question whether the hyperactivity of the cardiovascular system in hyperthyroidism is due to sensitization of the system to the effects of endogenous catecholamines, has been raised by several groups of authors, as reviewed by Harrison.(125) The suggestion has been prompted mainly by the similarity of manifestations of thyrotoxicosis and an infusion of epinephrine. One approach to the problem has been the use of the beta-adrenergic blocking agents, nethalide and propranolol to try and establish to what extent beta-receptor-mediated effects contribute to the pathophysiological state.

Howitt et al(129) demonstrated that the effects of intravenous propranolol are greater in hyperthyroid subjects than in euthyroid controls, implying that there is increased sympathetic activity in hyperthyroidism. Their conclusions are based on observations of heart rate, cardiac output, stroke volume, systolic ejection rate, aortic max dp/dt, left ventricular stroke work, stroke power, pressure-time-per-minute and ejection time. These authors had previously shown that after intravenous propranolol the heart rate of hyperthyroid patients in sinus rhythm or with atrial fibrillation, though reduced, remained elevated above the level attained by giving the same dose to normal subjects.(128) It was concluded that the residual tachycardia was due to the direct effect of thyroxine. The same conclusions have been reached by Wilson et al using

nethalide in hyperthyroid patients,[138] Wilson et al using propranolol in triiodothyronine-induced hyperthyroidism[139] by Cairoli and Crout[140] using propranolol in the unanaesthetized rat after thyroxine-induced hyperthyroidism and by Goodkind with reference to heart rate, peak systolic left ventricular pressure and LVmax dp/dt in the open-chested guinea-pig.[114] Two of these groups of authors[139,140] established that propranolol exerted effective adrenergic blockade in the hyperthyroid state by demonstrating effective blockade of the action of exogenous isoproterenol. Our own observations confirmed this point with respect to isoproterenol.

The effect of propranolol on myocardial contractility in the hyperthyroid state as measured in the intact animal, has not been reported.

In the experiments summarized in Fig.29 it is evident that the same relationships can be demonstrated for LVmax dp/dt in the hyperthyroid dog. The effect of propranolol was increased significantly after induction of hyperthyroidism and in no instance did it reduce the resting value to the levels obtained in normal animals. A substantial fraction of the increased level of contractility in the hyperthyroid dog is therefore not mediated through stimulation of beta-receptors by endogenous catecholamines, even though the activity of these appear augmented, and is attributable to a direct effect of the hormone.

4. <u>Thyroid status and responses to catecholamines</u>.

The synthetic beta-adrenergic stimulant isoproterenol was used to evaluate the responses of the heart in terms of rate and

contractility in normal, hypothyroid and hyperthyroid dogs. Although not a naturally-occurring catecholamine, its use has definite advantages. It is a relatively pure beta-stimulant and its effect on aortic pressure in the dose range used in these experiments is minimal. Vagal inhibitory reflexes are therefore not significantly elicited by large alterations of pressure as is the case with norepinephrine and epinephrine. Curves with highly significant linear regression on dose were obtained for heart rate, cardiac output and LVmax dp/dt. Isoproterenol is metabolized by the same pathways as the naturally-occurring catecholamines.(141)

The effect of induction of the hypothyroid state was to profoundly lower the entire dose-response curve for all haemodynamic measurements without a significant alteration in the slope of the curve. The maintenance of the same slope may, however, be due to the larger absolute amounts of isoproterenol administered to the hypothyroid and myxoedematous animals, all of which had gained excessive amounts of weight and the doses were calculated on a weight basis. In one severely myxoedematous animal it was, however, possible to obtain four studies without significant change in weight over a six month period. The curves obtained during the myxoedematous state clearly had a reduced gradient in comparison to the curves obtained during thyroid replacement.

The hearts of these animals can therefore respond to the stress of beta-adrenergic stimulation with loss of normal dose-response relationships only in the severely myxoedematous state. The entire response occurs at a much lower level of contractility.

Since myocardial oxygen consumption correlates linearly with LVmax dp/dt[142] this finding may explain the beneficial effects of induced hypothyroidism in patients with severe angina pectoris and congestive cardiac failure, first proposed by Blumgart et al[143] recently reviewed by Andrus.[144] The heart of such a patient, from the haemodynamic point of view, maintains its ability to respond to stress, but operates at a much reduced level of contractility and oxygen consumption with some attenuation of the responses to beta-adrenergic stress. In man, however, in contradistinction to the dog, the hypothyroid state is complicated by the early development and exacerbation of coronary atherosclerosis.

In the hyperthyroid animals, the opposite effect was seen. The dose-response relationships for LVmax dp/dt and heart rate were entirely disturbed by the administration of thyroxine for one week. This occurred in addition to a massive elevation of the level of contractility at which the heart functions. The dose-response curve now had a convex configuration instead of the strictly linear form in the euthyroid state. The plateau occurred at higher dose levels. On the other hand a marked increase in responsiveness to isoproterenol could be demonstrated at very low levels of the drug, levels at which the response during the euthyroid state was minimal or absent. This is in direct support of the observation by Riggs et al[146] in the dog, using epinephrine infusions. The use of atropine revealed that the plateau of the curve was not the result of failure on the part of the contractile mechanism, but of a powerful buffering action by the vagus nerve. The same conclusion was reached by Riggs et al and lends support to the suggestion of Hoffmann et al[147]

that the vagal inhibitory influences can be increased by catecholamines in the hyperthyroid state. It is also noteworthy that Beznak found the capacity of the heart to perform work greatly increased in thyroxine-treated rats.(148) These observations stand in sharp contrast to those of Whitehorn et al(149) who showed decreased contractile capacity in ventricular muscle from rats treated with thyroid, but these authors used an in vitro system only with isolated strips of muscle.

Our experimental findings are in direct opposition to those of Margolius and Gaffney(150) and van der Schoot and Moran(151) who found no alteration in responsiveness to catecholamines with alteration in thyroid state in dogs. As is evident in our experiments involving triidothyronine, the hearts of our animals responded briskly and responses in contractility could even be demonstrated in the absence of changes in heart rate. It is our belief that the difference between our work and their experiments is the absence and presence of, firstly, pentobarbital anaesthesia and, secondly, of extensive surgical intervention e.g. an open-chest preparation v.s. an intact conscious animal. We furthermore measured LVmax dp/dt directly whereas these authors measured peripheral arterial pressure and heart rate.

An illustration of the variable effects of pentobarbital on heart rate and myocardial contractility is provided in Fig.32. Observations of LVmax dp/dt and heart rate were obtained in a dog during euthyroid state and after one and three weeks of thyroxine treatment. Percentage changes in LVmax dp/dt are conspicuously greater than in heart rate. The dog was then given atropine; the influence on contractility was minimal, but heart rate increased further. Following atropinization to

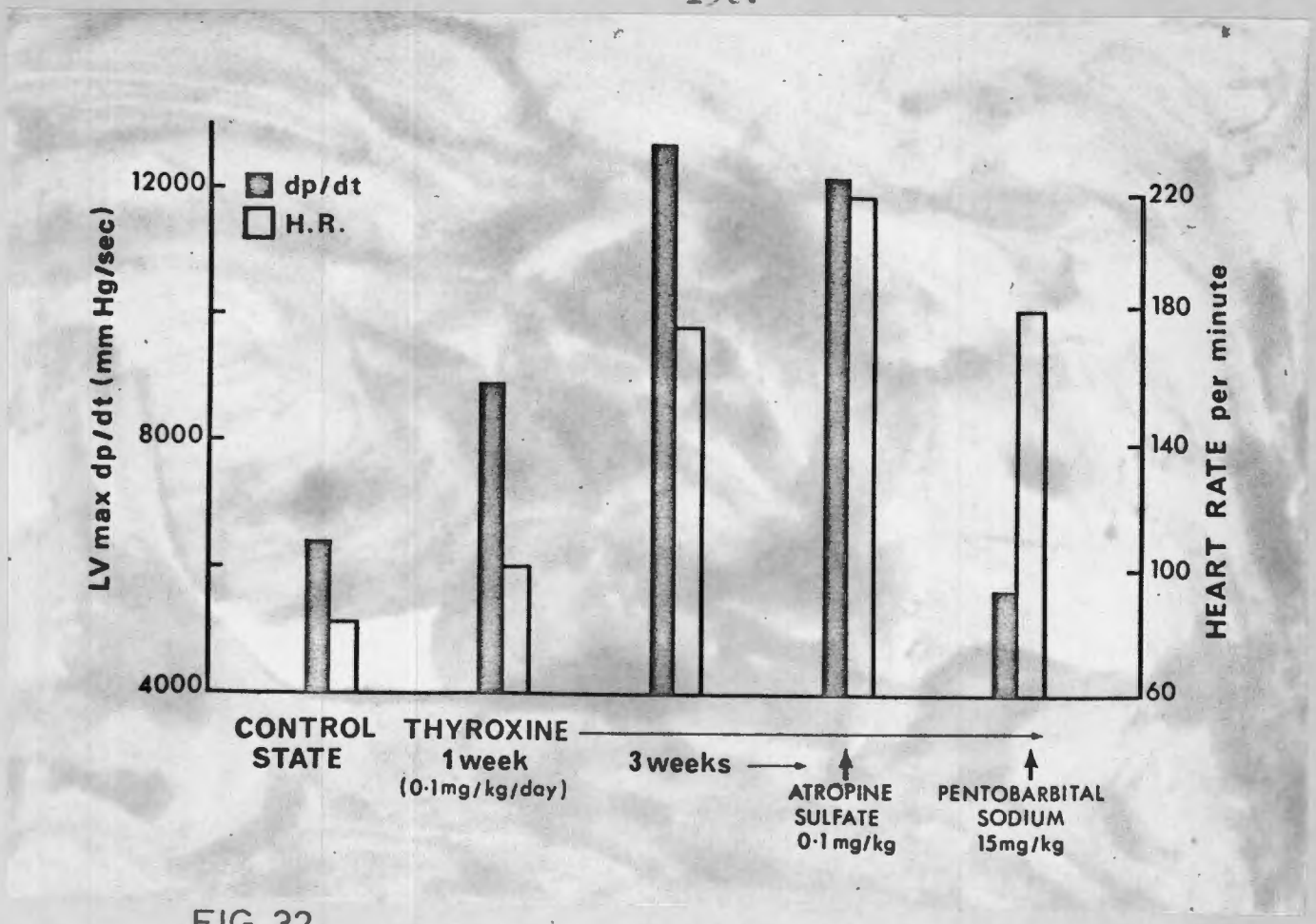


FIG. 32

preclude any mediation of effects through the parasympathetic system, pentobarbital was given 25 mg/kg by intravenous injection, a dose sufficient to induce light surgical anaesthesia. The disparity of effects on heart rate and myocardial contractility are striking. Pentobarbital has also been shown to act at various autonomic reflex levels.(152)

It remains difficult to interpret these data in terms of whether "responses" to catecholamine secretion are increased or decreased by alteration of thyroid status in these animals, since from this study it appears that the main effect of the hormone is to establish the level of contractility at which the heart operates, rather than alter the responses at a particular level. Our findings therefore do not help to resolve the contradictory conclusions of Brewster et al,(153) Schneckloth et al,(154) Murray and Kelley,(155) Hoffmann et al(147) who all

found increased "sensitivity" to injected catecholamines in hyperthyroidism, and Hoffmann et al[147] and Scheckloth et al[154] who demonstrated decreased responses in hypothyroidism on the one hand with Margolius and Gaffney,[150] van der Schoot and Moran,[151] Hess and Shanfield[156] and Aoki et al[157] who found that alterations in thyroid state did not alter responses to catecholamines. Most of these investigators, however, relied on measurements of peripheral arterial pressure and heart rate to arrive at their conclusions; weight alterations were in no instance corrected for in experiments involving hypothyroid subjects. Our findings and conclusions correspond more exactly with those of Leak and Lew[158] who concluded that responses to catecholamines remain intact in hypothyroidism until severe myxoedema develops, and are altered with disturbed dose-response relationships in the hyperthyroid state.

The finding that triiodothyronine will augment responses to isoproterenol without a demonstrable effect on resting haemodynamics and level of contractility, lends further support to this contention. The principal effect again was found to be an elevation of the level at which responses occurred. The occurrence of the effect within 15 minutes corresponds favourably with the demonstration by Kleinfeld et al[159] of an effect of triiodothyronine on the function of the frog's heart in 30 minutes. The fact that an effect could be demonstrated on contractility without an effect on heart rate strongly suggests that the action of the hormone is mediated not by the beta-receptor but by a mechanism operating nearer the contractile element.

5. The action of thyroid hormone on the heart.

A direct effect of thyroid hormone on the heart rate was demonstrated by Lewis and McEachern[160] who in 1931 showed that the tachycardia of thyrotoxic rabbits persisted in the isolated perfused hearts and in isolated atria. They also made the significant observations that the rise in pulse rate of their rabbits could be detected as early as 24 to 48 hours after commencement of thyroxine administration. Thyroxine added to the perfusing medium had no effect on normal atria. Priestley, Markowitz and Yater[161] demonstrated this same effect in isolated hearts of dogs and rabbits indicating that the effect was independent of an intact nervous supply to the heart. The possible role of significant amounts of free catecholamines, derived from the traumatized atrial tissue, could not be excluded though. These authors also performed the study on the transplanted heart of the dog, the data of one experiment only, however, being presented. Administration of thyroxine intravenously to the recipient animal resulted in a marked increase in rate of contraction of the transplanted puppy-heart which had beaten at a regular slow rate prior to the thyroxine injection. The increase in rate of the transplanted heart was noted to be far greater than that of the host animal suggesting that not only was the initiation of the tachycardia independent of the nervous system but in fact counteracted by intact innervation. Again the role of circulating catecholamines could not be assessed.

Markowitz and Yater[162] used cardiac tissue entirely devoid of nervous elements in an attempt to further resolve the problem. Myocardial cells from 48 hour old chick embryos were

cultured artificially for several days, displaying rhythmical contractions. These cells would not respond to the administration of epinephrine or acetylcholine, but administration of thyroxine to the culture medium induced a striking increase in rate of contraction, which was not reversed by acetylcholine. Although these authors indicated the purity of their thyroxine preparation and controlled their experiments in paired fashion, they omitted a control experiment with the alkaline solvent of their thyroxine. Since the volume of their culture medium was so small, a significant change in pH may have occurred and the effect of this was not assessed. Wollenberger[163] has recently reported similar experiments and demonstrated a chronotropic effect for l-triiodothyronine. The effect on his chick-embryo ventricular-cell preparations came on minutes after administering the hormone and was lost within five minutes of washing away the hormone.

The possible role of the nervous system in the mediation of the effects of thyroid hormone on heart rate received its main support from the work of Brewster et al[153] who studied the effect of total sympathetic blockade by epidural injection of procaine in normal and thyrotoxic dogs and found the heart rate of the two groups identical after the blockade. From this observation these authors concluded that the tachycardia of thyrotoxicosis was entirely mediated via the sympathetic nervous system. Their preparation was complicated; not only were the dogs anaesthetized with pentobarbital (their euthyroid animals had average heart rates of 170 beats per minute), but the animals were then subjected to thoracotomy as well as sympathetic blockade by injection of a large amount of procaine. The findings

of these authors have not been duplicated using a less extensively manipulated preparation and their data thus far stand unsupported. In the spinal cat, Benfey and Varma[164] recently observed that myocardial force and heart rate were greater in triiodothyronine-treated animals than in normal.

A similar approach to define the role of the sympathetic system has been the use of selective sympathetic blockade, using the beta-adrenergic blocking agents, nethalide and propranolol. As discussed earlier the majority of all authors are in agreement on both the points that the fraction blocked by these agents and the residual fraction are both increased in hyperthyroidism. Our own experiments have shown that these generalizations are also applicable to myocardial contractility in the intact dog.

Guanethidine, an agent that interrupts norepinephrine release at sympathetic neuro-effector junctions[165] in addition to depletion of norepinephrine stores at these nerve endings[166] was shown to have striking effects on, and may in fact correct the signs of thyrotoxicosis.[167] Gaffney et al[168] found that the drug could completely inhibit the rise in resting pulse rate and exercise-induced tachycardia of triiodothyronine-induced hyperthyroidism. Barker and Makiuchi[169] compared the effects of guanethidine and bretylium on thyroxine-induced tachycardia in rats. Bretylium acts like guanethidine, but does not deplete catecholamine stores. The effects of the two agents were identical and unlike the findings of Gaffney et al, did not succeed in blocking completely the chronotropic effect of thyroxine. Further support for the conclusions of Barker and Makiuchi is provided by the findings of de Groot et al[127] who used the ganglion-blocking agents, trimethaphan camphorsulfonate and guanethidine and found

that these agents did not restore the isometric contraction period and the duration of ventricular ejection in thyrotoxic subjects to normal.

Reserpine has for a long time had a place in the symptomatic treatment of thyrotoxicosis and thyroid crisis. Bray[170] attempted to define the role of the autonomic nervous system in the maintenance of the tachycardia in thyroxine-treated rats by the use of this drug. Thyroidectomized and thyroidectomized-adrenomedullated rats were used. Triiodothyronine administration six weeks after the surgical procedures, at which time adrenal regeneration was complete but endogenous epinephrine production absent, produced a significant increase in heart rate and B.M.R. in both groups. Reserpine in doses assumed to produce maximal norepinephrine depletion, prevented the increase in heart rate, but not the increase in B.M.R. induced by triiodothyronine. Reserpine administration to the thyroidectomized-adrenomedullated animals resulted in the death of many animals; this could be prevented by the daily administration of epinephrine. Triiodothyronine could raise the depressed heart rates of this group (thyroidectomized-adrenomedullated) to normal, but not higher. Bray suggests that a system - specific catecholamine - thyroid interaction occurs, proportional to the degree to which the system concerned is normally under autonomic control. This is supported by his findings that B.M.R. and oxygen consumption were not affected by catecholamine depletion in the triiodothyronine-treated animal, whereas heart rate was greatly. Atropine resulted in complete reversal of the reserpine-induced bradycardia in the triiodothyronine-treated animals. The heart rates

obtained after vagal blockade in these triiodothyronine-reserpinized rats were not different from those of animals treated with triiodothyronine but not reserpine. No data are supplied for animals treated with reserpine and atropine only and it is thus not possible to assess the influence of the parasympathetic system separately from the triiodothyronine effect. This study was performed in the intact, ether-anaesthetized rat.

Thier et al[171] conducted a similar study on isolated rat atria obtained from euthyroid, hypothyroid and hyperthyroid rats with and without superadded reserpine treatment. Their findings, supported by later experiments[172] showed that reserpine treatment did not abolish the differences in heart rate between the various groups. Their findings stand in contrast to those of Mendelsohn[173] who found that pretreatment with reserpine could prevent the tachycardia induced by thyroid feeding, except that in Thier's work the rats were rendered hyperthyroid by intraperitoneal injection of thyroxine and the variable of gastrointestinal absorption was eliminated.

The contradiction apparent in the findings of Bray and Thier et al in the ability of reserpine to normalize the heart rate of the hyperthyroid animal may be important. The work of Bray was performed in the intact animal, that of Thier had heart rate assessed in the isolated atrium. An obvious conclusion is therefore that an intact nervous system may be important; not only the sympathetic system, but particularly the parasympathetic system, since atropinization removed any differences in heart rates in the study of Bray between animals treated with reserpine and those not so treated.

The possible role of the autonomic nervous system in the resting tachycardia of experimental hyperthyroidism has been investigated by Cairoli and Crout.(140) These authors, working with rats, obtained heart rates in the conscious animals by electrocardiography. Propranolol, chlorisondamine and atropine were used in control and reserpine-treated euthyroid and hyperthyroid animals. Blockade of beta-adrenergic receptors produced less cardiac slowing in thyroxine-treated than in normal rats. Atropine subsequently administered, produced a sharp rise in rate in both hyperthyroid and euthyroid groups, suggesting that the vagal effect on the sino-atrial node is not particularly abnormal in the hyperthyroid rats. In these unanaesthetized rats, the fall in heart rate produced by chlorisondamine was the same in both euthyroid and hyperthyroid animals. The heart rates of thyroxine-treated rats thus responded to a ganglion-blocking drug and to the combination of propranolol and atropine in a manner similar to that of the euthyroid controls. However, a disparity in resting heart rate between thyroxine-treated and control animals is routinely observed by all investigators under all experimental conditions. These authors therefore concluded that the resting heart rate of the thyroxine-treated animal is modulated by autonomic mechanisms in a relatively normal fashion, but the high baseline rate is sustained by a non-neural process. Our own conclusions with reference to myocardial contractility are identical with those of this former group of authors.

These authors proceeded to investigate the interaction between the autonomic nervous system and the effects of reserpine treatment. Treatment with reserpine in hyperthyroid rats resulted in progressive lowering of the pulse rate to levels not

different from the levels attained in normal controls. They thus confirmed the earlier observations of Bray.[170] These reserpine-treated animals were then treated first with propranolol to block any residual effect of beta-receptor stimulation and 30 minutes later atropine was given. Propranolol induced a further fall in rate in both groups; atropine caused a marked increase in heart rate such that the original disparity in resting rates, before reserpine treatment, was restored. These findings strongly suggest that reserpine can normalize the heart rate in thyroxine-treated rats only if vagal function is intact. This fact, emerging from this study, may explain the apparent contradiction in the results of the findings of Bray[170] and Thier.[171]

An additional observation made by these authors was the fact that the differential effect of reserpine on heart rate in euthyroid and hyperthyroid animals could only be documented in the unanaesthetized animal. When the rats were given pentobarbital and then manipulated surgically the effect of reserpine was no longer visible, resembling the effect of atropine administration. This observation correlates well with our contention expressed earlier that observations on heart rate in anaesthetized animals are invalid if used to distinguish between thyroid states in experimental animals. The effects of anaesthesia are so variable that no conclusions can be drawn with any confidence.

The action of reserpine in hyperthyroid animals has therefore resulted in attention being focussed on the parasympathetic system as well as on the sympathetic system in thyroid disorders.

Reserpine appears to effect its rate-lowering effect by a mechanism involving the parasympathetic system in addition to its better known effect of catecholamine depletion. This focus on the parasympathetic system emphasizes our findings in the myxoedematous dog studied with atropine.

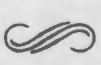
The studies of Thier, Bray and Cairoli are definitive, well-controlled and their results uniformly support the hypothesis that the intrinsic rate of the hyperthyroid state is set by an action of thyroid hormone directly on the pacemaker cells, rather than by a totally indirect action via the nervous system. Their findings suggest that the intrinsic rate of the heart is determined by a direct action while oscillations about this set level are modulated by the autonomic nervous system, including free catecholamines.

The recent findings of Buccino et al,[115] published since the commencement of this project, and ours suggest that the same generalizations apply to contractility. Thyroid hormone exerts a profound influence in determining the level of contractility, with its important implications for oxygen consumption, by a direct action on the contractile mechanism or its immediately associated metabolic processes. Our experiments with triiodothyronine confirm the earlier findings that the hormone acts to alter responsiveness to beta-adrenergic stimulation, but since the effect on contractility can be divorced from effects on rate, suggest that the action is not at the level of the beta-adrenergic receptor. Our experiments have in addition established the fact that the increase in contractility in hyperthyroidism is greater than can be accounted for by an autoregulatory increase associated

with the increase in heart rate.

The magnitude of the effect of the hormone on myocardial contractility may explain not only the beneficial effects of induced hypothyroidism on patients with angina pectoris but may also explain the high incidence of cardiac disease and failure, apart from rhythm disturbances, recently emphasized by Sandler and Wilson[94] in patients with clinical hyperthyroidism, especially in patients with meagre metabolic signs of hypersecretion of the hormone.

Recent investigations have shown that the alterations in myocardial contractility occur independently of changes in cardiac norepinephrine and high-energy phosphate stores.[115] A direct effect of thyroid hormone has been shown to occur at the level of mechano-chemical coupling with a decreased efficiency in the conversion of chemical energy to mechanical work in the myocardium of hyperthyroid subjects.[174] The possibility has recently been raised that thyroid hormone may act through the adenyl-cyclase-cyclic 3'5' - adenosine monophosphate system.[175] If, as has been suggested, adenyl cyclase is in fact the beta-receptor,[176] an attractive theory for the localization of thyroid hormone-catecholamine interaction in this complex system presents itself, but remains as yet unproven.



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SUMMARY.

The purpose of this thesis is to examine the influence of the thyroid status of an animal on the contractile state of its myocardium. This is prompted by the question whether primary myocardial actions of thyroid hormone contribute to the pathogenesis of the haemodynamic syndromes encountered in hypothyroid and hyperthyroid heart disease. Since myocardial performance was assessed by direct measurement of myocardial contractility, the principles of cardiac muscle physiology that underlie the definition of myocardial contractility are described in detail. The factors known to act as determinants of the contractile state of the myocardium are reviewed as well as the methods of assessment of myocardial contractility, with particular reference to the intact animal. A method is outlined whereby insight can be gained into the force-velocity relations of the intact heart by the measurement of the maximal rate of rise of pressure in the isometric phase of left ventricular contraction (LVmax dp/dt). Simultaneous measurements were, however, also obtained of the more traditional criteria of ventricular function, the relationships between stroke volume, work, power and rate of ejection versus the end-diastolic pressure, based on the Frank-Starling principle. Since the measurements were obtained in conscious animals sedated lightly with morphine, percutaneously-inserted catheters were necessary. The limitations of catheter-manometer systems in the faithful recording of cardiovascular

phenomena are discussed and an outline given of the method followed for the assessment of the frequency-response characteristics of such a system. A direct comparison of the catheter-manometer system with an intraventricular manometer system is described to establish the adequacy of performance of the system used for the recording of the frequency-dependent measurement of LVmax dp/dt.

LVmax dp/dt, stroke work, stroke power and systolic ejection rate were measured in normal, hypothyroid and hyperthyroid dogs. The hypothyroid state was obtained by treatment with I^{131}; the hyperthyroid state by subcutaneous injections of l-thyroxine. Correlation of LVmax dp/dt with thyroid status could be demonstrated, with LVmax dp/dt revealing greater quantitative changes than the other measures of contractility. The contribution of the increased heart rate with its associated autoregulatory increase in contractility in the hyperthyroid animals was assessed by obtaining rate-contractility curves by artificial right atrial pacing before and after the administration of thyroxine. The influence of alterations in thyroid status on the myocardial responses to beta-adrenergic stress was investigated by infusions of isoproterenol. It could be shown that the hormone determines the level of contractility at which the response occurs. In a grossly myxoedematous dog a loss of responsiveness could be shown; in hyperthyroid animals an increase in responsiveness could be shown to occur at low doses of isoproterenol while at higher doses complete loss of the dose-response relationships occurred, in addition to the massive elevation of the curve. Propranolol was used to demonstrate an increased state of beta-adrenergic stimulation, in addition

to a direct effect of the hormone on the muscle, independent of the beta-adrenergic receptor. A similar action of l-triiodothyronine was demonstrated fifteen minutes following its administration intravenously. At this stage an inotropic effect independent of a chronotropic component could be demonstrated.

The results are discussed in the light of findings of other workers, both recent and earlier. Emphasis is devoted to several authors who have recently provided evidence that supplements the conclusions reached in these experiments. These aspects of thyroid hormone action are reviewed and their implications in the physiology of the myocardium and pathophysiology of thyroid heart disease discussed.

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A P P E N D I X.

TABLE 1 - STATISTICAL ABBREVIATIONS.

Σ = Summation sign.

$\int$ = Integration sign.

N = Number of observations in a group.

X = Independent variable.

Y = Dependent variable.

$\bar{X}$ = The mean value for the group of X.

$\bar{Y}$ = The grand mean of the dependent variables.

x = Deviation from the mean = $X - \bar{X}$.

d.f. = Degrees of freedom.

S.E. = Standard error of the mean.

t = Student's t-distribution; i.e. the deviation of the estimated mean from the population mean.

F = Variance ratio = $\frac{\text{Mean square between classes}}{\text{Mean square within classes.}}$

Regression Analyses:

b = Sample regression coefficient = $\frac{\sum xy}{\sum x^2}$ where

x = Deviation of X from $\bar{X}$.

and y = Deviation of Y from $\bar{Y}$.

$\hat{Y}$ = Calculated values for Y.

d_{yx} = Deviation from regression = $Y - \hat{Y}$.

$\sum d_{yx}^2$ = Sum of squares of deviations from regression.

S_{yx}^2 = Mean square deviation from regression.

S_b = Sample standard deviation of the regression coefficient.

Ti = ΣX within a single distribution.

TABLE 2 - HAEMODYNAMIC MEASUREMENTS AT REST.

Section A - Euthyroid Group:

Dog No.	LVmax dp/dt (mm Hg/sec)	Heart Rate (per min)	Cardiac Index (ml/min/m^2)	M.A.P. (mm Hg)	LVEDP (mm Hg)	E.T. (msec)
1.	4640	72	-	-	-	-
2.	4640	66	-	-	-	-
3.	4370	64	2489	84	15.1	230
4.	4420	72	3766	107	9.5	230
5.	4120	76	2955	103	5.5	180
6.	4800	60	3232	110	8.6	200
7.	4160	64	3146	114	8.8	190
8.	5100	56	3885	114	7.1	210
9.	5000	80	3829	127	17.1	220
10.	5910	96	4704	125	9.5	220
11.	4930	75	3250	108	7.3	200
12.	4940	82	3472	119	9.3	180
13.	6100	82	2948	110	9.0	200
14.	4360	94	3912	79	10.8	200
15.	5100	90	3671	104	7.9	200
16.	6350	80	3491	116	6.6	200
17.	6490	96	4072	104	6.7	200
18.	6400	82	3846	100	7.2	200
19.	5000	80	3829	127	17.1	220
20.	6100	82	2970	110	9.0	200
21.	5100	90	3672	104	7.9	200
22.	6400	102	4272	104	6.2	200
23.	6400	82	3847	100	7.2	205
24.	5660	58	3240	100	6.0	210
25.	7200	72	4613	119	8.2	200
Σ X	133,690	1,953	83,111	2,488	207.6	4,695
$\bar{X}$	5,347	78.1	3,613	108	9.02	204.1
S.E.	175.0	2.5	113.1	2.47	0.67	2.72

Section B - Hypothyroid Group:

Dog No.	LVmax dp/dt (mm Hg/sec)	Heart Rate (per min)	Cardiac Index (ml/min/m^2)	M.A.P. (mm Hg)	LVEDP (mm Hg)	E.T. (msec)
1.	3200	46	2350	103	14.0	210
2.	3300	66	2450	120	15.6	240
3.	3300	70	1731	103	9.1	210
4.	3340	48	1436	102	8.4	190
5.	3430	90	2846	93	10.5	210
6.	2930	64	1763	108	8.1	200
7.	3780	70	3154	125	12.5	220
8.	2600	68	1496	85	6.0	200
9.	3710	80	2555	118	8.6	220
10.	2800	66	1742	102	5.2	190
11.	3740	68	2894	86	9.7	240
12.	3300	84	1341	115	15.0	210
13.	3660	84	3232	127	9.6	210
14.	3770	84	1780	109	10.0	210
15.	2760	45	1757	102	10.7	210
16.	2460	42	1555	80	4.8	220
17.	2950	60	2205	91	11.3	230
ΣX	55,030	1,135	36,287	1,769	169.1	3,620
$\bar{X}$	3,237	66.7	2134.5	104.1	9.94	212.9
S.E.	103.3	3.6	151.5	3.40	0.76	3.51

Section C - Myxoedematous Group:

1.	2040	44	961	94	10.4	210
2.	2210	38	1084	87	11.0	230
3.	1970	50	1105	67	6.5	230
4.	2100	44	1109	110	12.0	220
5.	2480	62	1146	92	11.0	230
6.	2260	45	942	70	10.2	250
ΣX	13,060	283	6,347	520	61.1	1,370
$\bar{X}$	2,176	47.1	1057.8	86.6	10.18	228.3
S.E.	74.7	3.35	34.8	6.56	0.78	5.42

TABLE 3 - RESPONSES TO ISOPROTERENOL INFUSIONS.

Section (i) Euthyroid and Hypothyroid groups:

A. Euthyroid Group (for values of LVmax dp/dt see pp. 109-112)

(a) Heart rate (per min).

Dog No.	Dosage in µg/kg/min			
	Baseline	0.05	0.10	0,20
1.	72	72	72	102
2.	66	66	84	108
3.	64	80	90	115
4.	60	66	84	102
5.	64	74	84	90
6.	56	66	80	98
7.	80	78	88	102
8.	96	92	96	106
9.	75	94	94	124
10.	82	74	86	110
11.	82	96	102	120
12.	94	94	98	120
13.	90	90	92	94
14.	80	76	82	84
15.	96	92	112	124
16.	82	96	106	126
17.	80	78	88	102
18.	82	96	102	120
19.	90	90	92	94
20.	102	92	112	124
21.	82	96	106	126
22.	58	68	86	94
23.	72	88	84	96
ΣX	1805	1914	2120	2481
$\bar{X}$	78.5	83.2	92.2	107.9
S.E.	2.7	2.3	2.2	2.7

(b) Cardiac Index (ml/min/m^2).

Dog No.	Dosage in µg/kg/min			
	Baseline	0.05	0.10	0.20
1.	1860	1790	1960	2560
2.	1460	1890	2540	3370
3.	2490	3010	4060	4670
4.	3230	3970	4650	7430
5.	3150	3520	4850	4570
6.	3890	3720	5010	5730
7.	3830	3560	4870	5780
8.	4710	4240	6010	6640
9.	3250	5340	4870	6390
10.	3470	2910	3410	5250
11.	2950	4160	5430	6030
12.	3910	3040	3220	3930
13.	3670	4090	5070	5020
14.	3490	3740	3240	3960
15.	4070	3760	4170	5270
16.	3450	4200	5380	7640
17.	3830	3560	4870	5780
18.	2970	4190	4460	6080
19.	3670	4090	5070	5020
20.	4270	3760	4170	5270
21.	3850	4200	5380	7640
22.	3240	3550	4640	6240
23.	4610	6450	5350	6060
ΣX	79,320	86,740	102,680	126,330
$\bar{X}$	3,448	3,771	4,464	5,493
S.E.	160.7	200.4	208.5	269.9

B. Hypothyroid Group:

(a) LVmax dp/dt (mm Hg/sec) - see page 110.

(b) Heart Rate (per min)

Dog No.	Dose of Isoproterenol in µg/kg/min Baseline	0.05	0.10	0.20
1.	66	72	78	96
2.	48	66	67	70
3.	90	90	90	92
4.	64	80	84	102
5.	70	76	88	90
6.	68	76	80	104
7.	80	84	90	100
8.	66	78	80	90
9.	68	60	64	80
10.	42	76	66	70
11.	60	78	82	88
ΣX	722	836	869	982
$\bar{X}$	65	76	79	89
S.E.	3.9	2.4	2.8	3.5

(c) Cardiac Index (ml/min/m^2).

1.	2450	3920	4730	5170
2.	1440	2250	2250	3250
3.	2850	2410	2570	3690
4.	1760	3150	2940	3670
5.	3160	3750	3970	4800
6.	1500	2060	2460	3620
7.	2560	3100	3170	3460
8.	1740	1660	1730	1990
9.	2890	4280	2490	2930
10.	1010	1880	2150	2090
11.	1430	1860	2220	2000
ΣX	22,790	30,320	30,680	36,670
$\bar{X}$	2,072	2,756	2,789	3,333
S.E.	219.8	279.2	264.9	318.6

C. Myxoedematous Group:

(a) LVmax dp/dt (mm Hg/sec) - See page 112.

(b) Heart Rate (per min)

Dog No.	Baseline	Dose in µg/kg/min 0.05	0.10	0.20
1.	44	44	46	48
2.	38	38	50	66
3.	50	52	54	58
4.	44	60	52	60
ΣX	176	194	202	232
$\bar{X}$	44	48	50	58
S.E.	2.4	4.8	1.7	3.7

(c) Cardiac Index (ml/min/m^2)

Dog No.	Baseline	Dose in µg/kg/min 0.05	0.10	0.20
1.	960	1930	1530	1500
2.	1080	1280	1460	1540
3.	1110	1570	1400	1680
ΣX	3150	4780	4390	4720
$\bar{X}$	1050	1590	1460	1570
S.E.	4 6	15 4	3 8	5 5

TABLE 3.

Section (ii) Thyroxine treated groups

(a) Before thyroxine treatment:

(i) LVmax dp/dt

Dog No.	Isoproterenol dose in µg/kg/min Baseline	0.05	0.10	0.20
1.	5000	5050	5470	6500
2.	6100	7750	8800	9360
3.	5100	7450	8230	8800
4.	6400	7310	7800	8840
5.	6400	8800	8370	8880
6.	5660	5900	6250	6440
7.	7200	7960	8240	10050
ΣX	41,860	50,220	53,160	58,870
$\bar{X}$	5,980	7,174	7,594	8,410
S.E.	296.6	483.7	469.1	527.4

(b) After one week thyroxine (0.1 mg/kg/day)

1.	11,420	13,100	11,690	11,600
2.	7,920	10,610	11,600	10,290
3.	11,200	14,750	15,340	15,750
4.	9,400	12,740	10,230	11,160
5.	8,830	10,930	12,840	13,920
6.	11,430	13,160	13,850	13,340
7.	10,970	12,360	12,690	13,040
ΣX	71,170	87,650	88,240	89,100
$\bar{X}$	10,167	12,521	12,606	12,729
S.E.	541.4	533.9	628.5	291.1

Ti	Before T4 v.s. After	Linear regression Before T4	Linear regression After T4	Quadratic regression Before T4	Quadratic regression After T4	Cubic regression Before T4	Cubic regression After T4
4186	-1	-7	0	+7	0	+3	0
5022	-1	-3	0	-4	0	-8	0
5316	-1	+1	0	-8	0	+6	0
5887	-1	+9	0	+5	0	-1	0
7117	+1	0	-7	0	+7	0	+3
8765	+1	0	-3	0	-4	0	-8
8824	+1	0	+1	0	-8	0	+6
8910	+1	0	+9	0	+5	0	-1
Divisor	56	980	980	1078	1078	770	770
Contrast	13,205	13,931	12,900	-3897	-11283	-1609	-4735
Sum of sq.	3113786	198033	169806	13957	118094	3363	29118
F.	248.3	15.8	13.5	1.1	9.4	0.3	2.3
P	<.0001	<.001	<.005	N.S.	<.005	N.S.	N.S.

(ii) Heart rate (per min)

(a) Before thyroxine.

Dog No.	Isoproterenol dose in μg/kg/min. Baseline	0.05	0.10	0.20
1.	80	78	88	102
2.	82	96	102	120
3.	90	90	92	94
4.	102	92	112	124
5.	82	96	106	126
6.	58	68	86	94
7.	72	88	84	96
ΣX	566	608	670	756
$\bar{X}$	80.5	86.8	95.7	108.0
S.E.	5.2	3.9	4.1	5.6

(b) After thyroxine (0.1 mg/kg/day for 1 week).

1.	102	120	140	156
2.	120	150	162	150
3.	102	114	136	150
4.	110	122	120	148
5.	100	114	132	140
6.	124	156	164	180
7.	114	122	128	142
ΣX	722	898	982	1066
$\bar{X}$	110.3	128.2	140.3	152.3
S.E.	3.6	6.5	6.3	5.0

	Before T4 v.s. After T4	Linear regression Before T4	Linear regression After T4	Quadratic regression Before T4	Quadratic regression After T4	Cubic regression Before T4	Cubic regression After T4
Divisor	56	980	980	1078	1078	770	770
Contrast	1118	1688	2478	-50	-714	98	-42
Sum of Sq.	22320	2908	6266	2	473	12	2
F	131.2	17.1	36.8	0.01	2.8	0.1	0.01
P	<.001	<.005	<.001	N.S.	N.S.	N.S.	N.S.

(iii) Cardiac output (ml/min)

(a) Before thyroxine

Dog No.	Isoproterenol dose in μg/kg/min. Baseline	0.05	0.10	0.20
1.	2440	2270	3100	3680
2.	1730	2440	2600	3540
3.	2130	2370	2940	2910
4.	2560	2250	2500	3160
5.	2180	2380	3050	4330
6.	1760	1930	2520	3390
7.	2490	3480	2890	3270
ΣX	15,290	17,120	19,600	24,280
$\bar{X}$	2,184	2,446	2,800	3,469
S.E.	128.2	183.6	96.2	172.2

(b) After thyroxine (0.1 mg/kg day for one week)

1.	1970	2790	2860	3470
2.	2440	3470	3330	2960
3.	2510	2960	3800	4370
4.	3040	2970	3060	3830
5.	3330	4250	4750	4770
6.	3190	4710	4670	4330
7.	3610	3970	3580	4860
ΣX	20,090	25,120	26,050	28,590
$\bar{X}$	2,870	3,589	3,721	4,084
S.E.	219.1	279.1	281.1	263.7

	Before T4 v.s. After T4	Linear regression Before T4	Linear regression After T4	Quadratic regression Before T4	Quadratic regression After T4	Cubic regression Before T4	Cubic regression After T
Divisor	56	980	980	1078	1078	770	770
Contrast	2356	7973	6737	315	-2530	223	-1298
Sum of Sq	99120	64866	46313	93	5939	64	2188
F	42.7	27.9	19.9	0.03	2.6	0.02	0.9
P	$<.001$	$<.001$	$<.001$	N.S.	N.S.	N.S.	N.S.

TABLE IV.

Dog No. G.500

1. RESPONSES IN EUTHYROID STATE:

	Isoproterenol dosage µg/kg/min.			
	0	0.05	0.10	0.20
LVmax dp/dt (mm Hg/sec)	6,400	8,800	8,370	8,880
Heart rate (per min)	82	96	106	126

2. RESPONSES AFTER ONE WEEK THYROXINE (0.1 mg/kg/day)

LVmax dp/dt (mm Hg/sec)	8,830	10,930	12,840	13,920
Heart rate (per min)	100	114	132	140

3. RESPONSES AFTER THREE WEEKS THYROXINE (0.1 mg/kg/day)

(a) Before atropine

LVmax dp/dt (mm Hg/sec)	12,670	12,840	12,930	14,300
Heart rate	176	174	178	182

(b) After atropine 0.1 mg/kg

LVmax dp/dt (mm Hg/sec)	12,200	15,080	17,650	18,390
Heart rate	218	194	200	208

TABLE V.

CONTRACTILITY AND HEART RATE BEFORE AND AFTER PROPRANOLOL.

LVmax dp/dt	Euthyroid state		After one week T4. 0.1 mg/kg/day	
Dog No.	Before	After 0.4mg/kg	Before	After
1.	5,000	2,180	11,420	5,140
2.	6,350	4,440	11,200	8,820
3.	6,400	5,160	9,400	7,880
4.	5,660	4,460	11,430	7,160
5.	7,200	4,760	10,970	7,220
Heart rate				
1.	80	80	102	96
2.	80	54	102	100
3.	102	78	102	74
4.	58	52	124	110
5.	72	56	114	82

TABLE VI.

1. EUTHYROID EXPERIMENTAL GROUP.

(a) LVmax dp/dt - before l-triiodothyronine.

Dog No.	Isoproterenol (µg/kg/min).			
	Baseline	0.05	0.10	0.20
1	4,640	5,160	5,320	6,630
2	4,640	6,540	7,480	9,160
3	4,370	5,400	6,540	6,800
4	5,910	6,080	6,800	7,940
5	4,930	5,560	5,720	5,920
6	4,940	5,300	5,470	5,650
With l-triiodothyronine 0.1 µg/kg/min.				
1	4,950	5,350	5,800	7,260
2	3,410	6550	9,210	10,250
3	5,380	6,520	7,050	7,820
4	6,670	6,810	7,210	8,300
5	5,670	6,300	6,030	6,570
6	6,420	6,600	6,750	7,140

(b) Heart rate - before l-triiodothyronine.

1	72	72	72	102
2	66	66	84	108
3	64	80	94	115
4	96	92	96	106
5	75	94	94	124
6	82	74	86	110
with l-triiodothyronine 0.1 µg/kg/min.				
1	60	66	84	100
2	66	102	100	114
3	72	82	118	136
4	80	84	92	118
5	58	62	84	128
6	72	76	78	98

2. EUTHYROID SOLVENT CONTROL GROUP.

(a) LVmax dp/dt before l-T3-solvent infusion.

Dog No.	Isoproterenol µg/kg/min.			
	Baseline	0.05	0.10	0.20
1	3,830	4,240	5,410	5,760
2	4,800	5,790	7,290	8,180
3	4,160	5,350	5,520	6,000
4	5,100	5,740	6,610	7,750
5	6,100	7,750	8,800	9,360
6	6,350	6,630	6,680	7,310
with l-T3-solvent infusion				
1	4,580	5,160	5,320	6,630
2	5,860	6,630	8,050	8,570
3	3,690	4,000	4,820	4,420
4	4,510	5,740	5,860	7,180
5	7,520	7,580	7,760	8,000
6	7,120	6,480	7,560	7,890
(b) Heart rate before l-T3-solvent infusion.				
1	78	90	108	112
2	60	66	84	102
3	64	74	84	90
4	55	66	80	98
5	82	96	102	120
6	80	76	82	84
with l-T3-solvent infusion				
1	66	72	72	102
2	56	70	82	104
3	74	84	100	94
4	50	56	60	92
5	72	76	86	90
6	84	84	80	78

3. d-T3 CONTROL GROUP.

(a) LVmax dp/dt before d-T3.

Dog No.	Isoproterenol (μg/kg/min).			
	Baseline	0.05	0.10	0.20
1	6,400	7,310	7,800	8,840
2	6,400	8,800	8,370	8,880
3	5,660	5,900	6,250	6,440
4	7,200	7,960	8,240	10,050
with d-T3 0.1 μg/kg/min.				
1	6,650	7,800	8,820	9,460
2	6,910	7,800	8,560	7,850
3	5,650	6,230	6,300	6,350
4	7,160	7,680	7,920	8,340
(b) Heart rate - before d-T3.				
1	96	92	112	124
2	82	96	106	126
3	58	68	86	94
4	72	88	84	96
with d-T3 (0.1 μg/kg/min).				
1	86	94	112	136
2	78	116	120	140
3	86	112	84	108
4	66	88	90	86

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4. HYPOTHYROID GROUP.

(a) LVmax dp/dt before l-T3.

Dog No.	Baseline	Isoproterenol 0.1 µg/kg/min. 0.05	0.10	0.20
1	3,300	3,800	4,310	4,860
2	3,340	3,820	5,470	6,040
3	2,930	3,820	3,860	4,700
4	3,780	5,020	5,550	7,630
5	2,600	3,860	4,520	5,720
6	3,710	4,890	5,560	6,720
with l-T3 0.1 µg/kg/min.				
1	3,290	5,000	5,470	6,260
2	3,860	4,680	5,220	7,350
3	3,300	4,100	4,260	5,430
4	5,150	6,930	7,750	8,920
5	3,010	3,950	4,920	7,000
6	4,310	5,580	7,070	7,690

(b) Heart rate - before l-T3.

1	66	72	78	96
2	48	66	67	70
3	64	80	84	102
4	70	76	88	90
5	68	76	80	104
6	80	84	90	100
with l-T3 0.1 µg/kg/min.				
1	60	75	76	84
2	40	50	72	80
3	64	72	80	92
4	68	75	90	100
5	60	68	76	82
6	76	76	102	106

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Renin in the Diagnosis of Renovascular Hypertension

Activity in Renal and Peripheral Vein Plasma

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Renin activity was assayed in plasma from the right and left renal veins and a peripheral vein in 47 hypertensive patients. Activity was equivalent in the two renal veins in 25 patients without renal artery stenosis; peripheral vein activity averaged 20% less. Ten of 17 patients with angiographic evidence of unilateral renal artery stenosis had significantly greater renin activity in the vein of the involved kidney; peripheral vein renin activity was equal to that from the vein of the uninvolved kidney. Two of five patients with bilateral stenosis had significant differences in renin activity between the two sides. The correlation between the results of the assays and results of surgery (14 cases) was excellent. Differential renal vein renin assays have diagnostic value in the determination of the functional significance of apparent unilateral renovascular disease.

Stenotic lesions occur in the renal arteries of normotensive as well as hypertensive patients.[1-3] A variety of methods has been used to detect renovascular disease and determine its physiologic significance in hypertensive patients. These methods include intravenous urography with modifications,[4] isotope renography,[5] the angiotensin amide infusion test,[6] renal angiography, split renal function studies,[7,8] determination of pressor activity of untreated plasma,[9] and renal biopsies.[10] Despite these procedures, the role of an angiographically visible stenotic lesion in the genesis of hypertension may remain in doubt.

The present studies, which extend our preliminary observations,[11] were undertaken to assess the value of comparative assays of renin activity in renal vein plasma from the two kidneys as a measure of the functional significance of an apparent renal artery stenosis. Renin activities of peripheral vein and renal vein plasma were also compared.

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Materials and Methods

Intravenous urography including films made at 15 and 60 seconds, renal arteriography, and assays of renin activity in plasma from the right and left renal veins were carried out in 47 hypertensive patients.

Renal vein catheterization was performed by the percutaneous transfemoral retrograde Seldinger technique without complications.[12] With the aid of television fluoroscopic control, a curved soft polyethylene catheter, with a side hole within 1 cm of the tip, was placed in each renal vein beyond testicular or ovarian branches. The final position was determined by visualization after injection of 3 ml of diatrizoate meglumine (Renografin-60). Before blood for assay was withdrawn, the catheter was thoroughly flushed with normal saline solution. The initial 5 ml of blood was discarded and a 25-ml sample was then collected in tubes containing sodium heparin and placed in ice. A similar sample of blood was collected from the brachial or femoral vein after collection of the second renal vein specimen. After centrifugation at 2,000 rpm for 15 minutes, the plasma was separated and frozen.

Renin activity in plasma was assayed by a modification of the method of Helmer and Judson.[13] After adjustment of pH to 5.5 with hydrochloric acid, plasma was dialyzed against cold running tap water for 24 hours. Isotonicity was restored by addition of sodium chloride and pH was readjusted to 5.5. The dialysate was frozen until immediately prior to assay when aliquots were incubated for one hour at 37 C. Amounts of 0.5 to 2 ml were added to a double-chambered muscle bath in which a spirally cut strip of rabbit aorta was suspended

in the inner chamber filled with Krebs bicarbonate solution. The buffer solution, with potassium concentration of 9.4 mEq/liter, was maintained at pH 7.4 by continuous aeration with 5% carbon dioxide and 95% oxygen. Temperature was maintained at 37 C by circulation of water through the outer chamber. Contractile responses, amplified nine times, were recorded by a lightweight, gravity-type aluminum heart-lever, writing on a carbon kymograph. Tension on the aortic strips was adjusted to 4 gm by counterweights. Freshly prepared synthetic asparaginyl-1, valyl-5 angiotensin II was used as the reference standard. Renin activity was calculated as millimicrograms of angiotensin II elaborated per milliliter of plasma in the hour of incubation.

The vasoconstrictor effect was identified as that due to angiotensin II, elaborated by the action of renin during incubation, by the following evidence: (1) recovery of the vasoconstrictor material in incubated dialyzed plasma after butanol-paper and filter-column chromatography by the method of Boucher et al[14]; (2) complete loss of vasoconstrictor activity by boiling the plasma for ten minutes before incubation (vasoconstrictor activity persisted when boiling was carried out after incubation); (3) complete destruction of vasoconstrictor principle by incubation with carboxypeptidase or lysed red blood cells; and (4) no loss of activity on prior exposure of the aortic strips to phentolamine hydrochloride, phenoxybenzamine hydrochloride, diphenhydramine hydrochloride, or atropine.

The recorded blood pressures in each patient represent averages of readings obtained on at least three days, when the patients were in the sitting position and the auscultatory method was used.

Results

The term renovascular disease is used in reference to angiographically visible disease of the main renal arteries or their primary branches.

Patients Without Renovascular Disease With Kidneys of Equal Size (Table 1).—Ten patients had essential hypertension, two in the malignant phase. One patient had membranous glomerulonephritis, one bilateral chronic pyelonephritis with azotemia, and one primary aldosteronism.

Renal angiograms were normal in all patients. Renography was done after administration of sodium iodohippurate I 131 (Hippuran-I-131) to five patients with essential hypertension; the curves were normal in four and bilaterally flattened in one (case 1).

Renin activity in plasma from one renal vein was close or equivalent to that in the opposite renal vein in every case. The mean renin activity per milliliter of plasma from the right renal veins was 14.6 mμg angiotensin II, that in the plasma from the left renal veins was 14.8 mμg. This difference between the two sides was not statistically significant. In no instance was there a difference of more than 4 mμg angiotensin II per milliliter of plasma. Peripheral vein renin activity averaged 10.7 mμg angiotensin II per milliliter of plasma. The difference between the activities in the renal and peripheral veins was statistically significant (paired sample t test, $P < 0.01$).

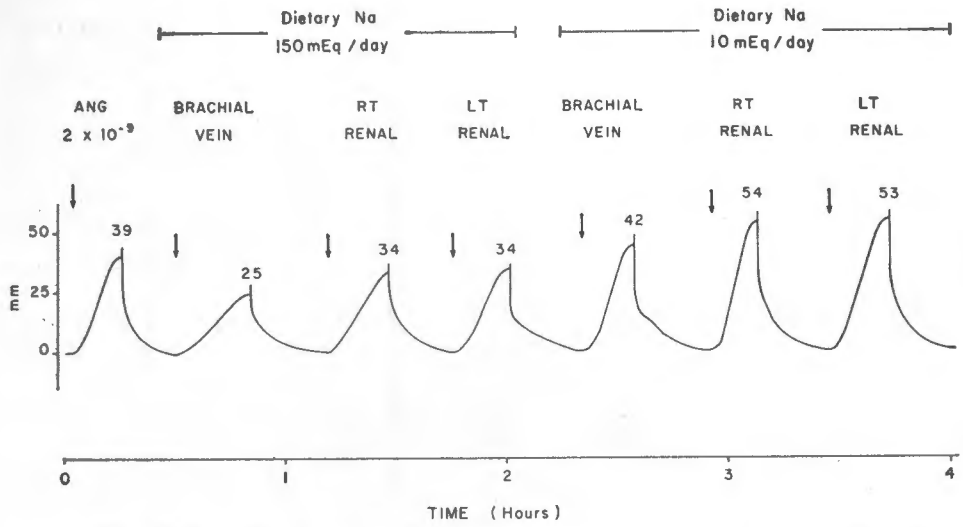
In one patient (case 12) renal vein catheterization was performed on two occasions, once during a period of high salt intake and again during a period of reduced salt intake. On a sodium intake of 150 mEq per day, the renal vein renin activity was 17 mμg angiotensin II per milliliter in both renal veins while the brachial vein level was 13 mμg. After decrease of dietary sodium to 10 mEq per day for four days, the renal vein renin levels increased to 27 mμg angiotensin II per milliliter on both sides and brachial vein plasma renin activity rose to 22 mμg. The aortic strip responses to the different plasmas in the two periods of study are shown in Fig 1.

In three patients brachial vein plasma renin

Table 1.—Plasma Renin Activity in Patients Without Renal Artery Stenosis and With Kidneys of Equal Size

Case No.	Age	Sex	Blood Pressure, mm Hg	Fundi*	Low Salt Diet or Diuretic	Plasma Renin Activity (mμg Angiotensin II/ml/hr) Right Renal Vein	Left Renal Vein	Difference Between Renal Veins	Peripheral Vein	Remarks
1	31	F	190/110	III	—	2	2	0	1	Essential hypertension
2	43	F	280/155	II	+	15	18	3	12	Essential hypertension
3	39	M	160/108	II	—	12	10	2	7	Essential hypertension
4	57	F	200/100	II	—	7	6	1	5	Essential hypertension
5	52	M	200/110	III	—	2	3	1	4	Essential hypertension
6	31	F	230/130	II	+	17	19	2	16	Essential hypertension
7	19	M	185/105	II	—	18	16	2	5	Essential hypertension
8	67	F	290/105	II	—	6	8	2	5	Essential hypertension
9	56	M	210/170	IV	+	44	47	3	33	Essential hypertension
10	46	F	260/160	IV	+	10	11	1	7	Essential hypertension
11	31	M	180/115	II	+	24	20	4	18	Membranous glomerulonephritis
12	50	M	200/120	II						Chronic bilateral pyelonephritis
					—	17	17	0	13	150 mEq Na in diet for 4 days
					+	27	27	0	22	10 mEq Na in diet for 4 days
13	28	F	160/100	I	—	3	3	0	2	Primary aldosteronism
					Mean	14.6	14.8	1.5	10.7	

*Retinopathy is graded according to the scale of Keith and Wagener.



1. Aortic strip responses to 2 ml of incubated dialyzed plaza (case 12) from brachial vein, right renal vein, and left renal vein after four days of a sodium intake of 150 mEq/day, at left; responses after four days of 10 mEq sodium daily, at right. Response to synthetic asparaginyl-1, valyl-5 angiotensin II (ANG) in a concentration of 2 mμg/ml of bath fluid is also shown. Number above each concentration refers to height of response in millimeters. Time scale reflects kymographic speed of 90 mm/hr.

activity was less than 5 mμg angiotensin II per milliliter. One of these patients had hypokalemic primary aldosteronism. Aldosterone excretion was normal in the other two.

Patients Without Renovascular Disease With Kidneys of Unequal Size or Shape (Table 2).—Of the 12 patients in this group, eight had differences greater than 2 cm in the bipolar diameters of their kidneys in the absence of a mass, two had cysts of the right kidney, and two had horseshoe kidneys. There was evidence of pyelonephritis in the smaller kidney in patient 16 and in both kidneys in patient 20. All had hypertension without angiographically visible renal artery stenosis. Only patient 21 had recently been treated with a diuretic or low sodium diet.

Right and left renal vein renin activities were approximately equal in all cases. Mean right renal vein plasma renin activity was 13.7 mμg angiotensin II per milliliter, the mean left renal vein renin activity was 12.8 mμg. The difference between the two sides was not statistically significant. Differences between the two sides ranged between 0 and 5 mμg. Activity in peripheral vein plasma averaged 9.9 mμg. The difference between the activities in the renal and peripheral veins was statistically significant ($P < 0.001$).

Nephrectomy was performed in one patient in this group, a 26-year-old hypertensive woman with a large difference in size between the two kidneys (case 14). Intravenous and retrograde urography showed no function on the left side. The difference in renal vein plasma renin activity between the two sides was 3 mμg angiotensin II per milliliter. Removal of the small hypoplastic left kidney did not reduce the blood pressure in a follow-up period of 18 months.

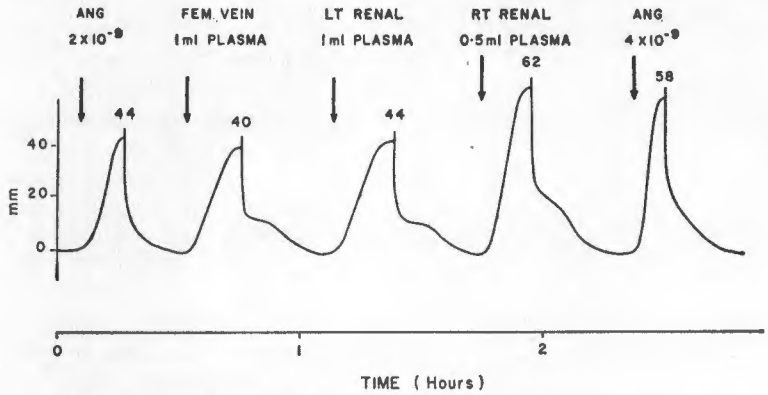
Patients With Unilateral Renovascular Disease (Table 3).—Fourteen of the 17 patients in this group had a stenotic lesion of a main renal artery. Three patients had lesions of a primary branch.

Ten patients had substantial differences in renal vein plasma renin activity between the two sides, differences greater than 10 mμg angiotensin II per milliliter (Table 3*a*). Only one person of these patients had a family history of hypertension. Presenting clinical manifestations included headaches, visual disturbances, and cerebrovascular, cardiac, and peripheral vascular disease. Two patients had grade IV retinopatny and three patients had grade III (Keith-Wagener scale). Patient 34 had an

Table 2.—Plasma Renin Activity in Patients Without Renal Artery stenosis, but With Differences in Size or Shape of Kidneys

Case No.	Age	Sex	BP	Fundi (K-W)	Low Salt Diet or Diuretic	Plasma Renin Activity (mμg Angiotensin II/ml/hr) Right Renal Vein	Left Renal Vein	Difference Between Renal Veins	Peripheral Vein	Remarks
14	26	F	160/95	I	—	10	7	3	9	RK* > LK by 12 cm. No BP change after removal of hypoplastic LK
15	55	F	200/100	II	—	16	16	0	8	LK > RK by 4 cm
16	33	M	160/95	II	—	11	12	1	11	RK > LK by 7.5 cm
17	69	F	300/135	III	—	26	21	5	19	LK > RK by 5 cm
18	48	F	200/120	III	—	9	11	2	7	LK > RK by 4 cm
19	45	M	150/90	I	—	12	9	3	8	LK > RK by 6 cm
20	57	M	230/130	II	—	10	8	2	6	LK > RK by 2 cm
21	56	F	230/120	II	+	19	22	3	14	RK > LK by 2.5 cm
22	57	M	190/110	II	—	9	11	2	7	Cyst of right kidney
23	68	F	200/100	II	—	18	16	2	15	Cyst of right kidney
24	56	M	230/140	III	—	10	8	2	4	Horseshoe kidney
25	37	F	160/100	II	—	14	13	1	11	Horseshoe kidney
						Mean 13.7	12.8	2.2	9.9	

*RK signifies right kidney; LK signifies left kidney.



2. Aortic strip responses to incubated dialyzed plasma (case 30) from femoral vein (1.0 ml), left renal vein (1.0), and right renal vein (0.5 ml) are shown in comparison with responses to 2 and 4 mμg/ml bath concentrations of synthetic angiotensin II.

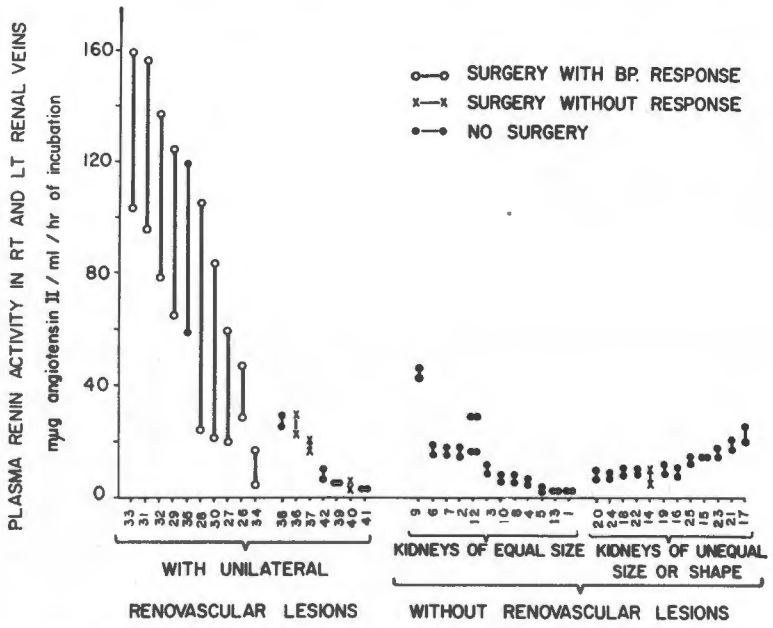
average blood pressure of 180/90 mm Hg and grade II fundi at the time of the renin study. In the previous four months he had been treated with methyldopa. Blood pressure before drug therapy averaged 210/110 mm Hg, and fundi were grade III at that time. Eight patients had differences of more than 1.5 cm in the bipolar diameters of their kidneys, four had delayed appearance of contrast material on the affected side on intravenous urography. The intravenous urogram was normal in all respects in two patients. Renograms made after administration of sodium iodohippurate I 131 were abnormal in six of seven patients. A patient with a branch lesion, case 25, had a negative test. Split renal function tests were positive in three of four patients with main renal artery stenosis and were negative, one by the Stamey and one by the Howard technique, in the two patients with lesions in primary branches.

The differences between the right and left renal vein renin activities in these ten patients ranged between 13 and 81 mμg angiotensin II per milliliter with a mean difference of 49.3 mμg. In the patient with a difference of 13 mμg, the left renal vein renin activity was four times that of the right side. The mean activity in the plasmas from the involved sides was 98.9 mμg angiotensin II, that in the plasmas from the uninvolved sides was 49.6 mμg. This difference was statistically significant ($P < 0.001$). The mean activity in the peripheral vein plasma in the eight patients in whom peripheral vein plasma was obtained was 42.4 mμg. There was no significant difference between the activity in the renal veins of the uninvolved side and that in the peripheral veins. The aortic strip responses to treated plasma from the three sites in a typical patient (case 30) of this group are shown in Fig 2.

Table 3.—Plasma Renin Activity in Patients With Unilateral Renal Artery Stenosis

Case No.	Age	Sex	BP	Fundi (K-W)	Low Salt Diet or Diuretic	Lesion	Plasma Renin Activity (mμg Angiotensin II/ml/hr) Renal Vein Involved Side	Renal Vein Uninvolved Side	Difference Between Renal Veins	Peripheral Vein	Surgical Procedure	Follow-Up, Mo	BP
(a) Patients With Substantial Differences in Renin Activity Between the Two Renal Veins													
26	57	M	200/110	II	+	Right main	47	29	18	27	By-pass graft	36	130/80
27	57	F	200/100	III	+	Right main	60	20	40	13	Nephrectomy	30	128/84
28	32	M	200/120	III	−	Left branch	105	24	81	28	Hemine-phrectomy	24	140/80
29	37	M	210/140	III	+	Left main	124	65	59	70	Nephrectomy	15	160/90
30	48	F	220/120	II	+	Right main	83	21	62	19	Nephrectomy	9	135/78
31	53	F	270/130	IV	+	Right main	157	95	62	80	Nephrectomy	18	180/100
32	57	F	280/135	IV	+	Left main	137	78	59	55	Nephrectomy	9	170/80
33	20	M	230/130	I	+	Left branch	160	103	57	...	Angioplasty	8	135/70
34	52	M	180/90	II	−	Left main	17	4	13	...	Nephrectomy	18	150/80
35	62	M	260/110	II	−	Right	119	57	62	47	Died before surgery		
						Mean	98.9	49.6	49.3	42.4			
(b) Patients Without Substantial Differences in Renin Activity Between the Two Renal Veins													
36	66	M	220/120	I	+	Left main	29	23	6	20	Nephrectomy	24	230/130
37	63	F	250/120	III	−	Left main	20	18	2	14	Nephrectomy	24	200/120
38	64	M	230/120	II	−	Left main	30	28	2	17	Exploration; no gradient	18	260/130
39	58	M	160/90	II	+	Right main	6	6	0	5	No surgery	...	...
40	13	M	140/90	I	−	Right branch	3	4	1	3	Angioplasty	24	140/90
41	52	F	170/120	II	−	Left main	3	3	0	...	No surgery	...	...
42	59	F	210/110	II	−	Left main	12	14	2	11	No surgery	...	...
						Mean	14.7	13.4	1.8	10.0			

Nine of these patients were treated surgically. Seven patients responded with continued normotensive blood pressures after surgery. A patient with a strong family history of hypertension (case 32) had marked improvement in level of blood pressure, disappearance of headaches, and regression of retinopathy from grade IV to grade II without further medical therapy. Patient 31 had a nephrectomy on the left side for occlusion of the left renal artery at the time of bypass for an occluded lower abdominal aorta. A cerebrovascular accident complicated the postoperative course. The blood pressure remained at normal levels for a period of three months, following which moderate hypertension developed without recurrence of the severe hypertensive retinopathy previously present. The blood pressures following surgery (Table 3) represent averages of at least three readings at the end of the indicated period of follow-up.



3. Renin activity in plasma from right and left renal veins in patients with and without angiographic evidence of unilateral renal artery stenosis. Results of surgery are indicated, also observations in patient 12 during periods of high and low sodium intake.

Seven patients had small or no differences in renin activity between the two renal veins (Table 3*b*). The differences ranged from 0 to 6 mμg angiotensin II per milliliter with a mean difference of 1.8 mμg. Renin activity in the vein of the kidney with the apparent stenosis averaged 14.7 mμg angiotensin II per milliliter; in the vein from the opposite side the average was 13.4 mμg; and in the peripheral vein plasma, 10.0 mμg. The difference between the two sides was not significant.

Four of these patients came to surgery. Unilateral nephrectomy was carried out in two without a reduction in blood pressure. A patient (case 40) with fibromuscular hyperplasia in a branch of the right renal artery had no change in blood pressure after angioplasty. Exploratory surgery was done on the fourth patient and no pressure gradient was found across the apparent stenosis.

The observations of plasma renin activity in the two renal veins in patients with unilateral renal artery stenosis and patients without renovascular disease are compared graphically in Fig 3. Two populations are apparent among the patients with renal arterial disease, a group with and one without large differences in renal vein renin activity. Renal vein renin activity in the group without differences between the two sides resembles that of patients without renovascular disease.

Patients With Bilateral Renal Disease (Table 4).—Four of the five patients in this group had atherosclerotic narrowing of both renal arteries, the fifth had bilateral fibromuscular hyperplasia. Two patients had large differences in renal vein renin activity between the two sides. Patient 43 had a renin activity difference of 60 mμg angiotensin II between the plasmas from the two renal veins. A renogram made after administration of sodium iodohippurate I 131 was abnormal bilaterally and the patient was azotemic. Reconstruction of the left renal artery was not technically feasible. Patient 44 had a positive split function test (Howard method). A surgical approach could not be undertaken because of other severe vascular disease.

Patients 45, 46, and 47 had differences of 2, 3, and 3 mμg, respectively, between the two sides.

Table 4.—Plasma Renin Activity in Patients With Bilateral Renal Artery Stenosis

Case No.	Age	Sex	BP	Fundi (K-W)	Low Salt Diet or Diuretic	Plasma Renin Activity (mμg Angiotensin II/ml/hr) Right Renal Vein	Left Renal Vein	Difference Between Renal Veins	Peripheral Vein	Remarks
43	65	M	210/105	II	+	43	103	60	63	Diffuse narrowing on right. Stenosis, left, at origin
44	48	M	210/130	III	—	6	22	16	. . .	Occlusion on left, with collaterals. Stenosis, right
45	41	F	230/110	II	—	24	22	2	15	Bilateral fibromuscular hyperplasia
46	50	M	190/70	III	—	14	11	3	10	Diffuse narrowing on left. Localized stenosis, right
47	51	M	270/170	III	+	7	10	3	6	Narrowing at origin of right. Left diffusely narrowed

Renograms made after the administration of sodium iodohippurate I 131 showed bilaterally flattened curves in the first two of these patients; the renogram was normal in the third. Patient 42 had a wide pulse pressure without diastolic hypertension despite angiographically severe bilateral renal artery stenosis. None of these patients was approached surgically.

Comment

Renin is a protein stored in the juxtaglomerular cells of afferent arterioles of the kidney. It is secreted in health as well as in disease. It is the initiating enzyme in a system that may act homeostatically to defend blood pressure and extracellular fluid volume in such conditions as salt depletion or hemorrhage, and may contribute to the pathophysiology of renal artery stenosis or certain edema states. Tobian has suggested that juxtaglomerular cells act as stretch receptors, stimulated to secrete renin in response to reduced pressure or volume in the renal arterial tree.[15] Small changes in mean renal arterial perfusion pressure have been shown to affect renin secretion.[16] The macula densa may also regulate renin secretion.[17]

Renin acts enzymatically on a tetradecapeptide substrate in the α_2 globulin fraction of plasma to elaborate a decapeptide, angiotensin I. Angiotensin I is not directly vasoconstrictor; it is split by a converting enzyme in plasma to an octapeptide, angiotensin II. This peptide has at least two actions, one as a vasoconstrictor, the other as a trophic hormone that stimulates the adrenal cortex to secrete aldosterone.[18,19] Angiotensinases are present in plasma and cells.[20]

Assays of Plasma Renin.—Assay of renin currently requires biologic techniques. Several methods are in use. In this study the method of Helmer and Judson[13] was used with modifications of the muscle bath and recording system. Catecholamines, histamine, and preformed angiotensin are removed by dialysis. To minimize angiotensinase activity in plasma, pH is adjusted to 5.5. Salt is added to permit the action of converting enzyme. The angiotensin II elaborated by the actions of renin and converting enzyme during incubation of the dialysate is determined by comparison of the vasoconstrictor effect of the incubated dialysate with the effect of known amounts of a synthetic angiotensin octapeptide standard on the same strip of rabbit aorta. Boucher et al[14] and Pickens et al[21] use the pressor response of a pentolinium tartrate treated or nephrectomized rat to determine the amount of angiotensin formed after incubation of the plasma following ion exchange chromatography or dialysis. In each of these methods the renin substrate is endogenous. By the method of Brown et al,[22] renin is first isolated by diethylaminoethanol cellulose-column chromatography; ox substrate is added, and the angiotensin formed during incubation is determined by a rat pressor assay. In the latter method, zero order kinetics have been demonstrated. These methods differ in several respects, particularly the handling of substrate and angiotensinase, the duration of incubation, and the method of determining the amount of angiotensin II elaborated. Yet, in both physiologic and disease states, results of these methods have been qualitatively similar although quantitatively different.

Previous studies in this laboratory were the first to show that peripheral and renal vein plasma renin activity increase sharply in normal and hypertensive subjects in response to mild salt depletion by a diuretic or low sodium diet.[23,24] The rabbit aortic strip method of assay was used. These observations have been confirmed by the other methods of assay.[21,25,26] Plasma renin activity is also increased in Addison's disease,[26] hemorrhage,[27] the erect posture,[28] pregnancy,[29,30] and the latter half of the menstrual cycle[30-32]; it is reduced in salt loading and primary aldosteronism.[25,26,28]

Renal Vein Renin.—In the present study renin activity was found equivalent in the right and left renal veins in hypertensive patients without renal artery stenosis, both in those patients with kidneys of equal size and in patients with unequal kidneys due to pyelonephritis, hypoplasia, or cysts. The largest difference in activity was 6 mμg angiotensin II per milliliter of plasma per hour of incubation. Patients with unilateral renal artery lesions fell into two groups, one with and one without distinct differences in plasma renin activity between the two sides. In the former group there was an average of a twofold difference in renin activity between the two sides. The smallest difference was 13 mμg angiotensin II per milliliter of plasma. The higher concentration of renin activity in venous blood of a kidney with functionally significant renal artery stenosis may reflect both an increase in secretion of renin and a reduction in blood flow in this kidney. Further studies are necessary to determine the relative contribution of these factors.

Nephrectomy or relief of the stenosis in patients with significant differences in plasma renin activity in the two renal veins reversed or strikingly modified the hypertension. Unilateral nephrectomy in patients with apparent stenosis, but without lateralizing differences in renin activity in the renal veins failed to affect the blood pressure. Patients with bilateral renal arterial lesions also fell into groups with and without substantial differences in renal vein renin activity between the two sides.

Peripheral Vein Renin.—Peripheral vein plasma renin activity in patients without functionally significant renal artery stenosis was about 20% lower than the activity in each renal vein. This difference was statistically highly significant. In patients with renal artery stenosis and a distinct difference in renin activity between the two sides, peripheral vein plasma renin activity was at approximately the same level as that in the renal vein plasma of the unaffected kidney. Determination of plasma renin activity in the three sites, a peripheral vein and the two renal veins, provides patterns of activ-

ity that separate patients with from patients without significant unilateral renovascular disease.

High peripheral vein plasma renin activity is not specific for renovascular disease since such states as salt depletion or pregnancy are also associated with high levels. The assumption of the erect posture for four hours induces a twofold increase in peripheral vein plasma renin activity. From the beginning to the end of the menstrual cycle, there can be an even greater increase. These observations indicate the need for control of these variables in the use of the peripheral venous plasma renin activity as a test for the detection of renovascular disease. In this series, while on normal salt intake, two patients with significant renal artery stenosis had renin activity levels of the same order as several patients without renovascular disease.

Current observations do not permit the conclusion that the high levels of renin activity account for the elevation in blood pressure in renovascular hypertension. Although salt depletion by a diuretic or low sodium diet is followed by a marked increase in renin and aldosterone secretion in hypertensive and normotensive patients, the blood pressure in the hypertensive patient gradually decreases while that of the normotensive individual is unaltered. In the course of normal pregnancy, the levels of renin activity and aldosterone secretion are high, but the blood pressure remains normal. Following experimental production of renal artery constriction in dogs, blood pressure and renin activity are both high initially, yet persistent elevation of blood pressure is accompanied by a gradual decline in renin activity.[33] Assessment of the activity of each of the components of the renin-angiotensin-angiotensinase system is required before conclusions can be drawn concerning relationships between the renin system and blood pressure. The recent demonstration by Smeby et al[34] of a phospholipid renin-inhibiting factor in plasma has introduced an additional factor that may be involved in the relationship between renin and blood pressure.

The Detection of Renovascular Hypertension.—In the search for patients with surgically curable renovascular disease among the large group of patients with hypertension, two approaches are used: screening to select patients for arteriography, and tests to determine whether a demonstrated stenotic lesion is the cause of the hypertension.

The yield of two screening procedures, modified excretory urography and renography after administration of sodium iodohippurate I 131 is high and when the two tests are used in combination, more than 90% of patients with renovascular hypertension can be recognized.[35] The occurrence of false negative intravenous urograms was noted in two patients in this study; the sodium iodohippurate I 131 renogram was abnormal in one. False positive tests are frequent with both procedures. The pressor response to intravenously infused angiotensin has also been suggested as a procedure to detect renovascular hypertension. Conflicting results have been reported.[36] Salt depletion and malignant hypertension must be excluded before this test is applied.

Proof of the functional significance of an angiographically visible renal artery stenosis may be obtained by the demonstration of increased water reabsorption by the involved kidney.[37] Hyperconcentration of contrast material and delayed disappearance after induced diuresis during urography reflect this pathophysiological disturbance. The split renal function techniques detect these phenomena by decrease in urine volume and sodium excretion[†] and increased urinary concentration of creatinine, inulin or *p*-amino hippuric acid (PAH)[*] on the affected side. These procedures are technically difficult and not devoid of risk. In a group of 100 cases, urine collections were unreliable in 17.[35] In our series, two patients with branch lesions of a renal artery had normal results from split function tests but abnormal findings from renal vein renin studies; surgery in each case was followed by a dramatic and persistent fall in blood pressure.

In this study of comparative renal vein renin assays in the determination of the functional significance of unilateral renovascular disease, false positives were not observed. Of the nine surgically treated patients with substantial differences in plasma renin activity in the two renal veins, seven became normotensive and two had marked improvement. False negative results were also not encountered. Observations of renal vein renin activity in renovascular disease have been presented in summary fashion by Judson and Helmer[38] and by Kirkendall et al.[39] The results of their studies and our own appear similar, despite differences in the methods of assay. Differential renal vein renin assay thus provides information of diagnostic value in the determination of the functional significance of angiographically visible renal artery stenosis.

Several aspects of the technique of renal vein renin studies require attention. A side hole, placed within 1 cm of the tip of the catheter, facilitates withdrawal of blood. The position of both apertures within the renal vein and distal to the testicular or ovarian branches should be established. Since the presence of iodine-containing dye has been observed to interfere with renin activity, the catheter should be flushed with saline and the first 5 ml of blood withdrawn should be discarded. Excess heparin may cause loss of renin activity; an amount not exceeding 10 units/ml of blood appears suitable.

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Generic and Trade Names of Drugs

Phentolamine hydrochloride—*Regitine Hydrochloride.*
Phenoxybenzamine hydrochloride—*Dibenzyline.*
Diphenhydramine hydrochloride—*Benadryl.*
Sodium iodohippurate I 131—*Hippuran, Hippuran-131, Hippuran-I-131, Hipputope.*
Methyldopa—*Aldomet.*
Diatrizoate meglumine—*Cardiografin, Gastrografin, Renografin.*
Pentolinium tartrate—*Ansolysen Tartrate.*
Angiotensin amide—*Hypertensin.*

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Antihypertensive Actions of Diuretics

Comparative Study of an Aldosterone Antagonist and a Thiazide, Alone and Together

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Hydrochlorothiazide in a daily dose of 150 mg and spironolactone in a daily dose of 400 mg (100 mg of the currently available preparation) were found to have equivalent antihypertensive effects—reducing basal blood pressure, decreasing the pressor response to levarterenol bitartrate (norepinephrine) and angiotensin amide, and increasing the depressor response to a ganglion-blocking agent, trimethaphan camsylate—in a double-blind study of 11 hypertensive patients. The two diuretics, when given together, appear to act independently and additively in their antihypertensive effects. Serum potassium level, lowered by hydrochlorothiazide, was in the normal range during administration of spironolactone alone or in combination with hydrochlorothiazide. Spironolactone can thus be a useful substitute for a benzothiadiazine derivative in the treatment of hypertension. The administration of a combination of these diuretics, a thiazide and an aldosterone antagonist, can render hypertension more readily manageable.

Benzothiadiazine derivatives and aldosterone antagonists, drugs that are diuretic by different mechanisms, are antihypertensive. Comparative studies of the antihypertensive effect of representatives of these two families of diuretics are few, and conflicting results have been reported.[1-5]

A double-blind study was undertaken to compare, in the same group of hypertensive patients, the effects of hydrochlorothiazide and spironolactone alone and together on basal blood pressure, serum electrolytes, plasma volume, and the blood pressure responses to infused pressor and depressor agents. The experimental design permits statistical approach to determine whether in the doses used hydrochlorothiazide and spironolactone have equivalent antihypertensive activity and whether additive, synergistic, or antagonistic effects occur when the two diuretics are given together.

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Patients and Methods

Eleven patients with sustained hypertension were studied; ten had essential hypertension and patient 4 had chronic glomerulonephritis. All had grade 2 (Keith-Wagner scale) fundal changes; none had congestive heart-failure or uremia. Sodium was not restricted in the diet, and there was no potassium supplementation during the periods of diuretic administration.

After a placebo had been administered for approximately four weeks, hydrochlorothiazide in tablets similar in appearance to the placebo was substituted in a dosage of 50 mg three times a day, for a period of four weeks. At this time, the placebo was resubstituted for a similar period, after which spironolactone was added in a dosage of 100 mg four times a day in the original form in which this agent was prepared for clinical use. (Subsequently another method of crystallization was adopted, resulting in increased gastrointestinal absorption.[6] Twenty-five milligrams of the currently available preparation is reported to be equivalent to 100 mg of the material used in this study.) After one month, hydrochlorothiazide was again substituted for the placebo in the same dosage as before, without knowledge by the patients of the change.

All blood pressures were determined by the same technician, using the auscultatory technique. She was unaware which agents were being administered. During each visit of the patients, blood pressures were obtained at intervals of a few minutes

for at least 30 minutes, with the patient in a recumbent position. The lowest two blood pressures obtained in a period of study were averaged to represent the basal blood pressure.

The pressor response to levarterenol bitartrate (norepinephrine) was determined during an intravenous infusion of 5.5μg of base per minute delivered by a constant-rate infusion pump for 10 minutes. After return of blood pressure to basal levels, an intravenous infusion of angiotensin amide was administered at the rate of 1μg/min for 10 minutes. Ten minutes later, trimethaphan camsylate was infused intravenously at the rate of 1 mg/min for 10 minutes. An average of the blood pressure values obtained at intervals of one minute from the sixth to the tenth minute of each infusion was taken to represent the response to the drug. Similar routines were followed during the initial placebo and all subsequent drug therapy periods.

Plasma volume was determined from two venous samples of blood drawn 12 and 18 minutes after intravenous injection of 5μc of ^{131}I-labeled normal human serum albumin. Serum sodium and potassium values were determined by flame photometry with lithium internal standard.

The data were analyzed statistically with a two-way analysis of variance.[7] In this analysis, the variation due to the four different periods of drug administration was partitioned according to a two-by-two factorial arrangement into the effects of hydrochlorothiazide, spironolactone, and interaction of the two drugs.[8] After statistical comparison revealed no difference between the two placebo periods, only the results of the first period of placebo administration were used in the analysis.

Results

The basal blood pressures in the different periods of placebo and drug administration for each of the 11 patients are shown in Table 1. The mean values for the basal measurements and the measurements obtained during levarterenol bitartrate, angiotensin amide, and trimethaphan camsylate infusions during the initial placebo and the subsequent periods of drug administration are shown in Table 2.

The two-way analysis of variance of basal systolic blood pressure is shown in Table 3. The subdivision of the variation due to treatments indicates that there are highly significant effects due to hydrochlorothiazide ($P<0.001$) and spironolactone ($P<0.001$). Furthermore, the data are consistent with the hypothesis that spironolactone and hydrochlorothiazide in combination produce an effect equivalent to the sum of the effects when each drug is given alone. Thus, the antihypertensive effect of hydrochlorothiazide was found unaffected by the presence or absence of spironolactone and vice versa, as evidenced by the lack of statistical interaction ($P>0.05$).

The analysis of variance was conducted on the basal levels and the levels during levarterenol, angiotensin, and trimethaphan infusions for both systolic and diastolic blood pressure. The probability values for the resulting statistical tests of significance on the hydrochlorothiazide effect, the spironolactone effect, and their interaction are shown in Table 4. The results are remarkably consistent with a highly significant hydrochlorothiazide and spironolactone effect ($P<0.001$) and persistently support the hypothesis of independent additive effects of these two drugs when given in combination. There is no evidence of drug synergism or antagonism,

Table 1.—Basal Blood Pressure During Administration of a Placebo, Hydrochlorothiazide, and Spironolactone*

	Basal Blood Pressure (mm Hg)				
Subject	Placebo	Hydrochlorothiazide†	Placebo	Spironolactone‡ and Placebo	Hydrochlorothiazide and Spironolactone
1	211/113	181/101	197/101	155/92	153/94
2	210/112	172/93	229/116	181/102	145/100
3	210/118	196/108	204/120	189/116	154/90
4	203/112	191/102	203/133	185/102	175/99
5	196/101	167/97	197/98	163/85	141/86
6	190/96	161/94	190/110	176/99	155/88
7	191/98	178/78	192/91	189/86	173/76
8	177/113	160/107	172/113	157/105	129/94
9	173/105	149/94	185/105	160/96	137/90
10	170/112	119/85	170/112	149/94	129/94
11	163/110	156/106	170/99	146/96	139/86
Mean	**190/108**	**166/97**	**192/109**	**167/98**	**148/91**

*The sequence of administration was placebo, hydrochlorothiazide, placebo, combination of spironolactone and placebo, and combination of hydrochlorothiazide and spironolactone.
†Dosage of hydrochlorothiazide was 50 mg three times a day.
‡Dosage of spironolactone was 100 mg four times a day.

Table 2.—Mean Systolic and Diastolic Blood Pressure During the Four Periods of Drug Administration

		Mean Systolic and Diastolic Blood Pressure (mm Hg)			
Agent Infused	No. of Patients	Placebo	Hydrochlorothiazide	Spironolactone	Hydrochlorothiazide and Spironolactone
None (basal value)	11	190/108	166/97	167/98	148/91
Levarterenol bitartrate	11	226/121	198/109	195/110	178/102
Angiotensin amide	9	232/130	200*/120*	203/117	180/106
Trimethaphan camsylate	6	171/103	141/91	140/88	119/79

*Based on seven patients.

Table 3.—Analysis of Variance on Basal Systolic Blood Pressure

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	Probability Value
Patients	10	10,156	1,015.6	...	...
Treatments	3	9,852	...	...	...
Hydrochlorothiazide effect	1	5,524	5,524	55.48	<0.001
Spironolactone effect	1	4,301	4,301	43.20	<0.001
Hydrochlorothiazide-spironolactone "interaction"†	1	28	28	<1	NS*
Residual	30	2,987	99.57	...	...
Total	**43**	**22,995**	...	...	...

*NS = not significant ($P>0.05$).
†The test of interaction examines whether the effect of the two drugs in combination differs from the sum of the effects of either drug alone.

Table 4.— Analysis of Variance on Level of Blood Pressure

	Probability Values							
	Basal		Levarterenol Bitartrate		Angiotensin Amide		Trimethaphan Camsylate	
Null Hypothesis Tested	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
No hydrochlorothiazide effect	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01
No spironolactone effect	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hydrochlorothiazide and spironolactone in combination is not different from the sum of the two alone	NS*	NS	NS	NS	NS	NS	NS	NS
Residual mean square (mm Hg)²	99.57	29.5	190.23	33.1	94.77	30.95	92.33	60.87
Degrees of freedom	30	30	30	30	22	22	15	15

*NS = not significant ($P > 0.05$).

Table 5.—Estimates of Mean Systolic and Diastolic Blood Pressure Change Due to Hydrochlorothiazide, Spironolactone, and Their Combination*

	Mean Effect† (mm Hg)		
Agent Infused	Hydrochlorothiazide	Spironolactone	Combination
None (basal value)	−22.4 ± 6.1 − 9.1 ± 3.3	−19.8 ± 6.1 − 8.4 ± 3.3	−42.2 ± 8.6 −17.5 ± 4.7
Levarterenol bitartrate	−21 ± 8.5 −10.3 ± 3.5	−24.1 ± 8.5 − 9.2 ± 3.5	−45.1 ± 12 −19.5 ± 4.9
Angiotensin amide	−29.1 ± 6.7 −10.5 ± 3.8	−22.3 ± 6.7 −13.2 ± 3.8	−51.4 ± 9.5 −23.7 ± 5.4
Trimethaphan camsylate	−28.5 ± 8.4 −10.7 ± 6.8	−23.2 ± 8.4 −13.2 ± 6.8	−51.7 ± 11.9 −23.9 ± 9.6

	Weighted Values (Means)†			
	Placebo	Hydrochlorothiazide	Spironolactone	Hydrochlorothiazide and Spironolactone
Effect of Hydrochlorothiazide	$\frac{-1}{2}$	$\frac{+1}{2}$	$\frac{-1}{2}$	$\frac{+1}{2}$
Spironolactone	$\frac{-1}{2}$	$\frac{-1}{2}$	$\frac{+1}{2}$	$\frac{+1}{2}$
Combination	−1	0	0	+1

*Estimates have 95% confidence limits.
†The mean effects are derived from the means in Table 2 by using the above weighted values.

Table 6.—Mean Serum Potassium and Sodium Levels, Plasma Volume During Four Periods of Drug Administration

	Mean Value in 11 Patients			
Measurement and Unit	Placebo	Hydrochlorothiazide	Spironolactone	Hydrochlorothiazide and Spironolactone
Serum potassium level (mEq/liter)	3.93	3.22	4.44	4.32
Serum sodium level (mEq/liter)	141.4	139.8	138.8	138.2
Plasma volume (ml)	3,004	2,747	2,619	2,495

Table 7.—Analysis of Variance on Other Effects

	Probability Values		
Null Hypothesis Tested	Serum Potassium Level	Serum Sodium Level	Plasma Volume
No hydrochlorothiazide effect	<0.001	<0.05	<0.001
No spironolactone effect	<0.001	<0.001	<0.001
Hydrochlorothiazide and spironolactone in combination is not different from the sum of the two alone	<0.001	NS*	NS
Residual mean square	0.090917	2.1763	27,921
Degrees of freedom	30	30	30
Units	(mEq/liter)²	(mEq/liter)²	(ml)²

*NS = not significant ($P > 0.05$).

ie, no statistical interaction ($P > 0.05$). Table 4 also indicates the residual mean square variance in blood pressure. The systolic as well as diastolic values are consistent for the different measurements.

Table 5 gives estimates of the mean effects on blood pressure and their 95% confidence limits for hydrochlorothiazide, spironolactone, and their combination during the basal state and during infusions of levarterenol, angiotensin, and trimethaphan. The results indicate that hydrochlorothiazide and spironolactone, in the doses used, each reduced systolic blood pressure an average of 20 to 30 mm Hg during the various experimental situations. Since the combined effect of these two drugs is independent and additive, the drugs together produce a mean systolic blood pressure decrease of about 40 to 50 mm Hg. The diastolic effects are similar for hydrochlorothiazide and spironolactone, with about a 10 mm Hg mean decrease for each and a mean decrease of about 20 mm Hg for the two together.

The effects of hydrochlorothiazide and spironolactone on serum potassium and sodium levels and plasma volume are given in Tables 6 and 7. Table 6 exhibits the means for each of the four periods of drug administration, and Table 7 summarizes the two-way analysis of variance for these data (following the format in Table 2). For serum potassium, the results demonstrate a highly significant decrease due to hydrochlorothiazide ($P < 0.001$), a highly significant increase due to spironolactone ($P < 0.001$), and a significant interaction of the two drugs. The latter indicates that, with reference to serum potassium, the effect of one of the drugs, spironolactone or hydrochlorothiazide, depends on whether the other is or is not present. In combination, there is an increase in serum potassium similar to the increase with spironolactone alone. For serum sodium, there is a significant decrease attributable to spironolactone ($P < 0.001$). With regard to plasma volume, a highly significant decrease can be attributed to each drug ($P < 0.001$). For both serum sodium and plasma volume, there is no evidence of interaction when the two drugs are given together.

Side effects were not observed during spironolactone administration. During hydrochlorothiazide therapy, gout developed in one patient. During administration of the two diuretics together, three patients had mild constipation and transient cramps of their calf muscles.

Comment

Four measures of antihypertensive action were assessed in this study: decrease in blood pressure

in the basal state, alteration in the pressor response to levarterenol bitartrate and, separately, to angiotensin amide, and increase in the depressor effect of a ganglion-blocking drug, trimethaphan camsylate. The statistical analyses indicate that in the doses used, hydrochlorothiazide and spironolactone had equivalent effects with reference to each of these measures. When the two diuretics were given together, their effects were additive.

The antihypertensive effects of each of these diuretics closely resemble those of a low sodium diet.[9,10] Similar responses have also been described after other diuretics, including mercurials, ethacrynic acid, and furosemide. Early in the course of salt depletion, plasma volume and cardiac output are reduced, and peripheral resistance is increased.[11] Blood pressure begins to fall after several days. After months of such therapy, plasma volume and cardiac output return to normal, and peripheral resistance is decreased.[12] The observation that diazoxide, a nonsulfamylated benzothiadiazine,[13] is both antihypertensive and salt-retaining has raised the question whether thiazides may be antihypertensive by a mechanism additional to that of salt depletion. Facts opposing this view are the remarkably similar antihypertensive effects of hydrochlorothiazide and spironolactone shown in the present study, the reversal of the antihypertensive effect of hydrochlorothiazide by administration of salt,[14] and differences in the actions of diazoxide and chlorothiazide when intravenously administered. Diazoxide reduces blood pressure within several minutes after intravenous administration[13]; it rapidly reduces peripheral resistance and increases cardiac output. In contrast, the antihypertensive effect of chlorothiazide does not occur before diuresis, and cardiac output is reduced, with an increase in peripheral resistance in the initial weeks of administration of this drug.

Previous studies in this laboratory,[15,16] confirmed by others,[17,18] show that salt depletion in normal and in hypertensive subjects is a strong stimulus to renin secretion. Plasma renin activity increases twofold to eightfold within 24 hours of administration of thiazide or phthalimidine diuretics. Angiotensin, elaborated by the enzymatic action of renin, can stimulate the adrenal cortex to secrete aldosterone. Aldosterone secretion is increased within a day of administration of a mercurial or a thiazide drug. The action of aldosterone at the distal renal tubules fosters the reabsorption of sodium and the excretion of potassium. This action of the renin-angiotensin-aldosterone system is homeostatic in the sense that it limits the saluretic effect of a diuretic such as hydrochlorothiazide; on the other hand, it fosters potassium depletion. The hypokalemic effect of hydrochlorothiazide in this study was marked. Five patients had decreases in serum potassium levels to less than 3 mEq/liter. The dose was large and was given continuously for a month, without restriction of sodium in the diet.

Spironolactone, competitively inhibiting the action of aldosterone at the distal renal tubules, reduces the renal secretion of potassium and favors an increase in serum potassium. In this study, there was a statistically significant rise in serum potassium concentration attributable to spironolactone. When spironolactone was given in combination with hydrochlorothiazide, serum potassium levels were about the same as when spironolactone was given alone. Drug interaction of an antagonistic nature was thus evident between spironolactone and hydrochlorothiazide during their combined administration with regard to serum potassium. Caution is indicated in the use of spironolactone in patients with chronic renal insufficiency. Hyperkalemia has been reported in several patients with azotemia during administration of spironolactone in combination with chlorthalidone or bendroflumethiazide.[3] Potassium supplements during administration of this drug should be avoided. Salt substitutes commonly contain potassium and may be an unrecognized source of this ion. Spironolactone has not been found to affect glucose or uric acid metabolism. It may cause gynecomastia, reversible upon withdrawal of the drug.

The use of an orally effective continuously active diuretic has become a cornerstone of the medical management of hypertension since the development of chlorothiazide ten years ago. Administered alone, a sulfamylated benzothiadiazine or a phthalimidine reduces systemic blood pressure approximately 10%. Such an agent also increases the hypotensive effects of other antihypertensive drugs. From the present and other studies, it appears clear that spironolactone can be used to obtain similar antihypertensive effects while avoiding the adverse effects of a thiazide on potassium, glucose, and uric acid levels. Indeed, the use of the two diuretics together, a thiazide and spironolactone, can provide a reduction in basal blood pressure equivalent to the sum of the reduction in basal blood pressure with either agent alone. In those patients in whom other drugs are required, a significant reduction in the dose and side effects may be obtained by the use of such combined diuretic therapy.

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Generic and Trade Names of Drugs

Hydrochlorothiazide—*Esidrix, Hydrodiuril, Oretic, Hydril, Aquarius.*
Angiotensin amide—*Hypertensin.*
Ethacrynic acid—*Edecrin, Endercin.*
Furosemide—*Lasix.*
Diazoxide—*Hyperstat.*
Chlorothiazide—*Diuril.*
Bendroflumethiazide—*Naturetin, Bristuron.*
Spironolactone—*Aldactone.*
Levarterenol bitartrate—*Levophed Bitartrate.*
Normal human serum albumin—*Albumisol, Proserum.*
Trimethaphan camsylate—*Arfonad Camsylate.*

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