Cardiac Failure in the Dog as a Consequence of Exogenous Hyperthyroidism

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ABSTRACT

Thirty dogs were made hyperthyroid by feeding them 1 g/kg USP thyroid powder or injecting 1.2 mg/kg of l-thyroxine/day. Seven of the 30 dogs used had surgically induced mild valvular lesions of the right heart to determine whether preexistent organic disease was a requisite to the induction of failure in hyperthyroidism.

At 2 to 27 months the animals were subjected to cardiac catheterization for measurement of cardiac work and metabolism in vivo. The animals were then killed and the levels of the high energy phosphate compounds creatine phosphate (CP) and the adenine nucleotides (ATP + ADP) in the heart were measured as an index of the net efficiency of the processes of oxidative phosphorylation.

By hemodynamic or histopathologic criteria, 13 dogs showed signs of failure, only 3 of which had preexistent valvular lesions. The failure was not accompanied by a decreased efficiency of oxidative phosphorylation. Net levels of CP and ATP + ADP in the myocardium were normal. Preliminary studies with sarcosomes isolated from 5 animals also revealed normal P/O ratios in 3 dogs with and in 2 without signs of cardiac failure. Liver mitochondria isolated from these same 5 animals all demonstrated decreased P/O ratios. Decreased myocardial extractions of both glucose and pyruvate occurred with failure, suggesting some degree of myocardial hypoxia with increased intracellular glycolysis. Areas of relative hypoxia may exist in a hypertrophied myocardium of the hyperthyroid animal.

ADDITIONAL KEY WORDS uncoupling of oxidative phosphorylation myocardial substrate metabolism sarcosomes liver mitochondria high energy phosphates thyrotoxic heart failure hemodynamics

Hyperthyroidism has long been recognized as one of the causes of cardiac disability in man. Although paroxysmal tachycardia, paroxysmal fibrillation, and extrasystoles are the more common manifestations, about 12% of the patients have persistent auricular fibrillation or cardiac insufficiency (1). Clinical studies of thyrotoxic cardiac insufficiency have shown that cardiac failure occurs with much greater frequency when the myocardium is damaged by previous disease or aging (1, 2). On the other hand, cases of congestive heart failure associated with hyperthyroidism in young and otherwise healthy individuals have been reported (3, 4). It has been contended (5-7) that uncoupling of oxidative phosphorylation may be evoked by excess thyroid hormone in the myocardium as in other tissues and induce failure. However, no data have been presented relevant to the effects of such biochemical deficiencies in the myocardium on the work performance of the heart.
CARDIAC FAILURE IN HYPERTHYROIDISM

The present studies were undertaken in large animals to allow a full description of the work function of the hyperthyroid heart and to determine how metabolic effects of excess thyroid hormone on the myocardium affect cardiac function, especially with regard to the production of cardiac failure.

Hyperthyroidism was induced by feeding or subcutaneously injecting large amounts of thyroid hormone (8). Cardiac function and metabolism were studied by cardiac catheterization. At the conclusion of these studies, the animals were killed and tissues analyzed for the high energy phosphate compoundscreatine phosphate (CP) and adenine nucleotides (ATP + ADP). Specimens were also removed from 5 of these animals for isolation of the heart sarcosomes and measurement of the net efficiency of oxidative phosphorylation as P/O ratios. Since it has been observed clinically that failure occurs with greater frequency where there is preexistent cardiovascular disease, 7 of the 30 animals studied had surgically induced mild valvular lesions of the right heart but were not in failure at the time the thyroid regimen was started. Preliminary reports of this study have been made (9, 10).

Materials and Methods

Thirty adult male dogs weighing between 13 and 25 kg were used. Seven of these had been operated upon to produce mild tricuspid insufficiency and pulmonic stenosis (TI/PS) by the methods of Hufnagel et al. (11) and Barger et al. (12). These dogs had shown no signs of congestive heart failure during the 5-27 months (mean was 15 months) that preceded thyroid treatment. There were no ascites, hepatomegaly, cardiomegaly or significantly elevated end-diastolic filling pressures in either chamber. The hyperthyroid state was induced by feeding the dogs desiccated USP thyroid powder (final dose, 1 g/kg/day) or by injecting 1-thyroxine (1.2 mg/kg/day). The schedule of hormone administration and the methods of determining the onset of hyperthyroidism have been reported (8).

Cardiac work and metabolism were measured by closed chest cardiac catheterization in 15 dogs before (3 with TI/PS) and in 26 dogs after (6 with TI/PS) the induction of hyperthyroidism. The dogs were sedated with morphine sulfate (1-1.5 mg/kg im) 30 min before being anesthetized with 1:1 Nembutal: Dial-urethane (0.25 ml/kg iv for normal dogs or 0.16 ml/kg iv for hyperthyroid dogs). In every case the desired surgical plane was reached by adjusting administration of the anesthetic to the individual animal. Catheters were placed in the pulmonary artery, coronary sinus, and femoral artery. Under fluoroscopy, pressures were measured in the right atrium, right ventricle, pulmonary artery and in the pulmonary-artery wedged position as well as in the femoral artery and coronary sinus by Statham strain gauge transducers amplified through a Sanborn Twin-viso cardiette. Blood samples were drawn simultaneously from the coronary sinus and femoral artery. Oxygen and carbon dioxide contents were analyzed by the method of Man-Slyke and Neill (13); glucose, according to Nelson’s modified method of Somogyi (14); lactic acid, by the method of Barker and Summerson (15); pyruvate, by the method of Friedemann and Haugen (16); FFA, using a modified method of Dole (17). Coronary flow was measured by the nitrous oxide saturation technique with nitrous oxide analyses of the blood done according to the method of Kety and Schmidt (18).

After catheterization, animals were sacrificed for the study of phosphate distribution and the measurements of oxidative phosphorylation of isolated sarcosomes. The chest was opened under positive pressure ventilation with oxygen and while the heart was in sinus rhythm, the apex of the heart was quickly removed to a Dewar flask containing dry-ice-acetone. The following phosphate moieties were measured by the method of LePage and Umbreit (19) and expressed as μmole/g wet weight: total acid-soluble phosphate, inorganic phosphate, creatine phosphate (CP), 7-min acid hydrolyzable phosphate of the barium insoluble moiety (ATP + ADP) as corrected for co-precipitated fructose-1,6-diphosphate (FDP). An aliquot of the dissolved barium precipitate was analyzed for FDP by the method of Boe and Papadopoulos (20). In one hyperthyroid animal the adenine nucleotides were evaluated by newer enzymatic methods (21, 22).

In 4 normal and 5 hyperthyroid dogs a second piece of myocardium was taken for the isolation of sarcosomes. Following the removal of the apex for phosphate assays while the heart was still beating, a 20-25 g piece which included both ventricles was transferred to a beaker of chilled sucrose-Versene (0.3 M and 0.001 M respectively). To increase the quantitative yield of sarcosomes from dog heart this tissue was first homogenized in a Waring blender for 20 sec with cooling of the blender vessel in a 2 C alcohol bath every 5 sec. The resultant 80-ml suspension was homogenized for 60 sec in four aliquots with a loose-fitting Potter-Elvejem homogenizer, again
with intermittent cooling. The isolation procedure was continued as outlined by Slater and Cleland (23). Measurements of oxidative phosphorylation were made using both the classical Warburg manometric technique and the polarographic technique of Chance and Williams as modified by Packer (24). The assays were done in a phosphate buffer at pH 7.4 with alpha-ketoglutarate as substrate and malonate to prevent further oxidation of succinate formed.

As a biological control of mitochondrial changes produced by excess thyroid hormone as administered here, similar studies were performed on mitochondria of liver of these same dogs isolated as described by Schneider and Hogeboom (25).

Autopsies were performed on all of the dogs in order to aid in the diagnosis of cardiac failure. The weights of the heart, liver, and lungs were expressed as grams per kilogram of ascites-free body weight. Histological examinations were made by Dr. John Kurtz, Chief Pathologist, St. Margaret's Hospital, Pittsburgh, Pennsylvania.

Cardiac function was assessed from calculations of the efficiency of minute work and from the relationship of stroke work to ventricular end-diastolic filling pressure. Oxygen consumption and cardiac output, determined by the Fick method, were related to surface area according to a revised formula for mongrel dogs described by Cowgill and Drabkin (26). In order to calculate the energy cost of the minute cardiac work \[
\frac{(\text{ml } O_2/100 \text{ g/min} \times \text{g heart} \times 2132)/1000}{\text{1000}}
\]
in the control or pre-thyroid state an estimate of the heart weight was necessary. This was obtained using the figure of 7.8 g/kg (sd ± 0.78), the average ratio of heart weight to body weight as determined in 33 normal dogs in this laboratory. The heart weight necessary for this calculation in the hyperthyroid state was obtained for each animal at autopsy.

Results

GENERAL FINDINGS

The animals developed clinical and laboratory signs of hyperthyroidism in from 1 to 3 months as described previously (8). Using the following criteria, cardiac failure was diagnosed in 13 of the 30 animals (43%): 1) reduced minute work efficiency, 2) reduced stroke work per unit ventricular end-diastolic filling pressure, and 3) gross or histopathological evidence of chronic passive congestion in the liver and lungs.

The co-existence of mild valvular lesions of the right heart in 7 animals did not appear to make them more susceptible to failure. Comparing equal groups of animals with normal hearts and those with mild TI/PS lesions matched for length of time on the thyroid treatment, the incidence of failure was the same.
Failure was not accompanied by signs such as anorexia, marked loss of body weight, marked increase in body temperature, or dehydration. Of the 3 animals that died 1 to 2 months after thyroid treatment, only one demonstrated the toxic sign of a sudden, marked loss of body weight. All 3, however, showed evidence of cardiomegaly, hepatomegaly, and histological evidence of chronic congestion in both lungs and liver consistent with the diagnosis of cardiac failure.

**PHYSIOLOGICAL FINDINGS**

On the basis of the above criteria of failure the hemodynamic and metabolic data are presented in three groups: Group I. Control; Group II. Hyperthyroid without failure; Group III. Hyperthyroid with failure. Since the effects of hyperthyroidism were the same in dogs with and without TI/PS lesions, no further subdivision of the groups has been made. The statistical significance of the mean differences between groups was expressed by the P values of Student's t test.

Hyperthyroidism in the dog, as in man, is characterized by a high cardiac output syndrome as illustrated by the data in Table 1. In the compensated state, Group II, this increased output was achieved with a moderate increase in heart rate and no disproportional increase in oxygen usage. Since the increase in external work performed was greater than the increase of oxygen usage, the mechanical efficiency of the heart was greater in the hyperthyroid than in the control state. Such an inotropic effect is also suggested by the increase in ventricular stroke work performed per unit end-diastolic filling pressure (Figs. 1 and 2).

In 10 animals, however, similar hemodynamic measurements showed a marked decrease in efficiency, being 30% lower than in the control state and 50% lower than in hyperthyroid animals of Group II described. Other hemodynamic signs of failure are illustrated in Figures 1 and 2. Hemodynamically, the most characteristic findings with failure were the marked tachycardia and the dramatic increase in oxygen usage.
PIATNEK-LEUNISSEN, OLSON

TABLE 2

Organ Weights in Dogs with Chronic Hyperthyroidism

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of dogs</th>
<th>Body (kg)</th>
<th>Heart (g/kg body wt)</th>
<th>Liver (g/kg body wt)</th>
<th>Lungs</th>
<th>Wall thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>17.1</td>
<td>7.8</td>
<td>25.4</td>
<td>8.2</td>
<td>6.0</td>
</tr>
<tr>
<td>SD ±</td>
<td></td>
<td>2.2</td>
<td>0.8</td>
<td>8.8</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td>0.6</td>
<td>0.3</td>
<td>2.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>II. Hyperthyroid</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>without failure</td>
<td></td>
<td>17.9</td>
<td>9.2</td>
<td>24.4</td>
<td>9.2</td>
<td>7.8</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>4.1</td>
<td>1.9</td>
<td>6.0</td>
<td>2.8</td>
<td>1.7</td>
</tr>
<tr>
<td>SD ±</td>
<td></td>
<td>1.0</td>
<td>0.5</td>
<td>1.5</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III. Hyperthyroid</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with failure</td>
<td></td>
<td>19.3</td>
<td>10.2</td>
<td>31.1</td>
<td>11.9</td>
<td>7.1</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2.9</td>
<td>3.1</td>
<td>9.5</td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td>SD ±</td>
<td></td>
<td>1.6</td>
<td>1.0</td>
<td>3.2</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RV = right ventricle; LV = left ventricle.

**PATHOLOGICAL FINDINGS**

Cardiomegaly was found in all hyperthyroid dogs (Table 2). However, measurement of the thickness of the ventricular walls demonstrated that in those dogs without evidence of hemodynamic failure (Group II) the hypertrophy was confined to the right ventricle. In those animals described as being in failure both the right and the left ventricles were significantly increased in wall thickness.

The signs of systemic congestion with failure in Group III were increased weights of the lungs and varying degrees of pulmonary congestion on histological examination. Although the mean weight of the livers was not significantly increased in failure, several individual dogs showed large increases in weight of this organ. Varying degrees of hepatic congestion were noted on microscopic examination in all animals in failure.

The mild degree of surgically induced valvular lesions of the right heart in the 7 dogs operated on was confirmed at postmortem examination. In 6 dogs, one of the lesser leaflets of the tricuspid valve was non-functional; in the seventh dog, chordae supporting the major leaflet had been severed. Cardiac failure was precipitated in this latter dog with thyroxine feeding and in 2 of the dogs with damage of the lesser leaflet. Ascites developed only in the dog with damage to the major leaflet. The stenosis of the pulmonary artery in the 7 animals was relatively slight. The circumference of the pulmonary artery was reduced only about 30%.

**METABOLIC FINDINGS**

We measured 1) the availability of utilizable substrates as expressed by the arterial level (micromoles/ml whole blood), 2) the ability of the heart to extract substrates as expressed by the extraction coefficient (arterio-venous difference/arterial level), and 3) the energy equivalent or the amount of oxygen accounted for by the simultaneously extracted substrates assuming that the substrates were completely oxidized. These data are presented for the carbohydrate substrates glucose, lactate, and pyruvate in Table 3 and for free fatty acids (FFA) in Table 4.

It would appear that the increased energy needs of the hyperthyroid heart that is not in failure are met by increased levels of blood glucose and FFA with corresponding increases in the myocardial extraction of these substrates (Tables 3 and 4). Interestingly, only
### TABLE 3

Myocardial Substrate Extraction in the Chronic Hyperthyroid Dog with and without Cardiac Failure

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of dogs</th>
<th>Glucose ( \text{A—V} ) μmole/ml</th>
<th>Lactate ( \text{A—V} ) μmole/ml</th>
<th>Pyruvate ( \text{A—V} ) μmole/ml</th>
<th>A—V ΔX 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>15</td>
<td>0.00 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.22 ± 0.06</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>II. Hyperthyroid</td>
<td>16</td>
<td>5.00 ± 0.03</td>
<td>0.18 ± 0.07</td>
<td>0.23 ± 0.06</td>
<td>27.0 ± 2.7</td>
</tr>
<tr>
<td>without failure</td>
<td></td>
<td>0.13 ± 0.03</td>
<td>0.15 ± 0.07</td>
<td>0.23 ± 0.06</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>III. Hyperthyroid</td>
<td>10</td>
<td>5.78 ± 0.03</td>
<td>0.14 ± 0.03</td>
<td>0.23 ± 0.03</td>
<td>26.0 ± 3.0</td>
</tr>
<tr>
<td>with failure</td>
<td></td>
<td>5.78 ± 0.02</td>
<td>0.14 ± 0.07</td>
<td>0.23 ± 0.06</td>
<td>26.0 ± 3.0</td>
</tr>
</tbody>
</table>

Art. μmole/ml = arterial level, micromoles/ml whole arterial blood; A—V/A = arterial-coronary sinus difference/arterial level. P: probability of significance of group mean difference from control as calculated by Student’s t test; only values of 0.05 or less are given.
TABLE 4

Effect of Chronic Hyperthyroidism in the Dog on Myocardial Metabolism of Carbohydrate and FFA

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>FFA</th>
<th>A — V</th>
<th>A — V × 100</th>
<th>Oxygen equivalent (%)</th>
<th>Carbohydrate</th>
<th>FFA + carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control*</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>.363</td>
<td>.089</td>
<td>27.2</td>
<td>41.3</td>
<td>49.0</td>
<td>92.5</td>
</tr>
<tr>
<td>SD ±</td>
<td></td>
<td>.146</td>
<td>.028</td>
<td>8.6</td>
<td>18.5</td>
<td>17.6</td>
<td>13.5</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td>.031</td>
<td>.006</td>
<td>1.8</td>
<td>4.1</td>
<td>3.8</td>
<td>3.2</td>
</tr>
<tr>
<td>II. Hyperthyroid without failure</td>
<td>4</td>
<td>.519</td>
<td>.115</td>
<td>23.7</td>
<td>63.0</td>
<td>40.9</td>
<td>100.4</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>.120</td>
<td>.037</td>
<td>11.7</td>
<td>21.3</td>
<td>44.0</td>
<td>46.0</td>
</tr>
<tr>
<td>SD ±</td>
<td></td>
<td>.060</td>
<td>.019</td>
<td>5.8</td>
<td>10.6</td>
<td>22.0</td>
<td>23.1</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td>.00</td>
<td>.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III. Hyperthyroid with failure</td>
<td>4</td>
<td>.318</td>
<td>.062</td>
<td>21.2</td>
<td>27.6</td>
<td>53.2</td>
<td>80.9</td>
</tr>
<tr>
<td>Mean</td>
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<td>.050</td>
<td>.033</td>
<td>12.7</td>
<td>12.0</td>
<td>14.8</td>
<td>5.1</td>
</tr>
<tr>
<td>SD ±</td>
<td></td>
<td>.025</td>
<td>.017</td>
<td>6.4</td>
<td>6.0</td>
<td>7.4</td>
<td>2.8</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td>.06</td>
<td>.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data compiled from 23 normal animals not used in the hyperthyroid study.
FFA = free fatty acids. Other abbreviations as in Table 3.

TABLE 5

Partition of Acid-Soluble Phosphorus in Heart Muscle from Normal and Hyperthyroid Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>TASP</th>
<th>Pᵢ</th>
<th>CP</th>
<th>ATP</th>
<th>FDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>7</td>
<td>42.9</td>
<td>7.1</td>
<td>3.1</td>
<td>4.6</td>
<td>1.15</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>6.1</td>
<td>1.4</td>
<td>0.9</td>
<td>0.3</td>
<td>0.40</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td>2.9</td>
<td>0.5</td>
<td>0.4</td>
<td>0.1</td>
<td>0.15</td>
</tr>
<tr>
<td>II. Hyperthyroid without failure</td>
<td>12</td>
<td>36.5</td>
<td>7.7</td>
<td>4.3</td>
<td>5.2</td>
<td>1.54</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.3</td>
<td>1.1</td>
<td>1.7</td>
<td>0.9</td>
<td>0.56</td>
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<tr>
<td>SD ±</td>
<td></td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td>0.3</td>
<td>0.16</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td>.05</td>
<td>.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III. Hyperthyroid with failure</td>
<td>7</td>
<td>36.5</td>
<td>7.9</td>
<td>4.3</td>
<td>5.2</td>
<td>1.32</td>
</tr>
<tr>
<td>Mean</td>
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<td>3.9</td>
<td>1.1</td>
<td>1.0</td>
<td>0.5</td>
<td>0.33</td>
</tr>
<tr>
<td>SD ±</td>
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<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>0.26</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td>.05</td>
<td>.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TASP = total acid-soluble phosphate; Pᵢ = inorganic phosphate; CP = creatine phosphate; FDP = fructose diphosphate; ATP = barium-insoluble 7-min hydrolyzable phosphate expressed as ATP. All values are μmoles per gram.

extraction of pyruvate was significantly decreased. In conjunction with this it may be noted that the level of fructose diphosphate, the phosphorylated intermediate of the glycolytic scheme, appeared to be elevated in these hearts (Table 5).

In the hyperthyroid dogs in cardiac failure the energy needs of the heart did not appear

Circulation Research, Vol. XX, February 1967
CARDIAC FAILURE IN HYPERTHYROIDISM

249

to be met by extraction of the substrates mentioned. The ability of the heart to extract both glucose and pyruvate was significantly decreased as shown by the decreased extraction coefficients. The 19% reduction in the extraction coefficient of lactate also supports the suggestion that an increased intracellular glycolysis may in part account for the reduced extractions of glucose and pyruvate. Again, the tissue level of fructose diphosphate was elevated above normal (Table 5).

The preliminary studies of FFA utilization (Table 4) suggested that there is also a decreased amount of FFA available to the myocardium for oxidation in hyperthyroid animals in failure as compared to those not in failure. The arterial levels of FFA and A-V differences were not elevated in Group III as had been observed with those dogs of Group II (Table 4). The reduced contribution of FFA to the oxidizable substrate pool was further manifested by calculation of the energy equivalents of the extracted substrates. In the hyperthyroid heart in failure the amounts of extracted glucose, lactate, pyruvate, and FFA could account for only 81% of the simultaneously extracted oxygen.

Tissue Findings

There was no evidence of uncoupling of oxidative phosphorylation in the myocardium of hyperthyroid dogs with or without failure. The levels of creatine phosphate (CP) and the combined adenine nucleotides ATP and ADP, measured as the 7-min hydrolyzable phosphate of the barium-insoluble fraction of an acid extract, were unchanged even in the animals with cardiac failure (Table 5). Although the levels of CP reported in Table 5 are much lower and the Pi higher than values obtained today by improved methods of tissue freezing, the comparisons made between the groups of this study are considered valid. Using newer enzymatic assays for the measurement of the adenine nucleotides in the heart of one hyperthyroid dog, levels comparable to those in normal dog heart tissue were found: ATP, 5.67 μmole/g wet weight; ADP, 0.68 μmole/g wet weight. Of the phosphate moieties examined, only the total acid-soluble phosphate was found to be significantly changed, with a reduction from 42.9 to 36.5 μmole/g. The increase in the level of fructose diphosphate, while not statistically significant, may offer further evidence suggesting that there is greater glycolysis in the hyperthyroid heart, as postulated from the decreased extraction of pyruvate in these hearts. Further studies are being planned to examine the glycolytic schema of the hyperthyroid heart.

Preliminary studies with sarcosomes isolated from hyperthyroid hearts, both with and without failure, also suggest that the process of oxidative phosphorylation is not uncoupled by the excess thyroid hormone. In 5 animals so examined, normal P/O ratios of 3.2 ± 0.1 were comparable to a mean of 3.5 ± 0.5 for preparations from normal dogs. These 5 dogs had been maintained for 8–27 months on the thyroid program, and it had been found that 3 were in failure when sacrificed, one with demonstrable ascites. There did not appear to be any "loose" coupling in the sarcosomes examined. On the addition of ADP the rate of respiration was stimulated approximately threefold, comparable to that found in normal dog heart preparations, and returned to a "before-ADP" rate with completion of the phosphorylation of the added ADP.

Mitochondria isolated from the livers of these same dogs did demonstrate inefficient oxidative phosphorylation. Compared to a normal mean P/O ratio of 3.3 ± 0.4 by Warburg assay, a mean of 2.3 ± 0.1 was found in preparations from the 2 hyperthyroid dogs without failure and a mean of 2.1 ± 0.6 from the 2 dogs with cardiac failure. Examination of the polarographic records of these latter two preparations also revealed some "looseness of coupling" in that the respiratory rate on addition of ADP was stimulated fourfold but on completion of ATP formation the rate remained elevated, being approximately twice that of the rate before ADP.

Discussion

This study has demonstrated that cardiac failure can result from hyperthyroidism alone in previously healthy dogs. Normal levels of
CP and the adenine nucleotides ATP and ADP found in the failing heart of the hyperthyroid dog suggest that the failure is not due to an uncoupling of oxidative phosphorylation. Although these levels per se do not exclude the possibility of inefficient oxidative phosphorylation, the finding of normal sarcosomal function as P/O ratios in preparations from 3 animals in failure tends to support such a conclusion. Only in 1 of the 3 preparations was any "looseness" of coupling noted, but even here a normal P/O ratio was obtained.

Ernster et al. (27) have reported that mitochondria of skeletal muscle from hyperthyroid patients also function with normal efficiency in vitro but with some loss of control. This loss of control was associated with a greater activity of mitochondrial ATPase. Wang and Benmiloud (28) have also described an increased activity of the sarcosomal ATPase of thyroxine-treated rats. On the other hand, increased mitochondrial or sarcosomal ATPase may simply be related to the increased turnover rates of the high energy phosphate compounds noted by Fleckenstein et al. (29) and may not be detrimental to the energy metabolism of the hyperthyroid heart. Further studies are required to correlate these biochemical changes with the work performance of the hyperthyroid heart.

Other aspects may be considered in attempting to understand the failure of the hyperthyroid heart. The decreased minute work and decreased efficiency in failure could be the hemodynamic result of the marked tachycardia, and a decrease in filling and ejection times relative to the time of isometric contraction (30). Since the latter requires the greater portion of metabolic energy, the metabolic cost could become so disproportional to the mechanical work performed that a decrease in efficiency would result. Also, the reduction in the percentage of time the hyperthyroid heart spends in diastole could serve to restrict the optimum distribution of coronary flow through the myocardium, since it is during diastole that the greater proportion of such flow occurs. Kirk and Honig (31) have shown that there is a non-uniform distribution of coronary flow through the different areas of the myocardium of a dog with a normal heart rate; extravascular pressure was greater and flow was less in the endocardium than in the epicardium at the height of systole. Oxygen tension was also lower in the deeper layers than in the more superficial layers of the myocardium of the normal dog heart. The hyperthyroid heart with a marked tachycardia and an increase in percentage of time spent in systole might be more affected by such nonuniform distribution of coronary flow. This, coupled with the increased oxygen requirement of all areas of the myocardium induced by the cellular effects of excess thyroid hormone, might then lead to areas of relative hypoxia within the myocardium to the detriment of adequate work function.

The reduced extraction of carbohydrate substrates (glucose, pyruvate, and possibly lactate) by the failing hyperthyroid heart described in this study would support the idea of a relative hypoxia in the myocardium. Increased rates of intramyocardial glycolysis with increased tissue levels of glycolytic end-products as pyruvate and lactate would then depress further extraction of such carbohydrate substrates from the circulating blood. Wollenberger et al. (32) have described greater increases in levels of the glycolytic products fructose diphosphate and lactate in the total myocardium of thyroxine-treated dogs as compared to control dogs subjected to reduced flow through the myocardium. Their findings also suggest that the hyperthyroid heart is biochemically more susceptible to myocardial ischemia.

Increases in circulating levels of FFA have often been associated with a catecholamine-stimulated lipolysis of adipose tissue. Thus, the lack of such increases in a hyperthyroid animal in which thyroxine catecholamine synergism has been reported to be exaggerated is surprising (33). As pointed out more recently, although the tachycardia of hyperthyroidism appears to be mediated by a hyperactive sympathetic system, metabolic effects do not seem to be so related. Bray (34)
has found that the oxygen consumption of the heart is still elevated in the hyperthyroid heart even after the effects of the catecholamines have been suppressed. Van der Schoot and Moran (35) found the contractility of the hyperthyroid heart to be decreased but still sensitive to the inotropic effects of additional catecholamines. It would appear that excess thyroid hormone does exert an independent effect on the myocardium at the cellular level, elevating the level of metabolism and effecting a decrease in myocardial contractility in some manner as yet undefined.

These preliminary studies demonstrating that oxidative phosphorylation of liver mitochondria was significantly depressed while that of the heart was not in the hyperthyroid dog were subsequently confirmed in studies with the rat by Lee (36) in this laboratory. The role that such a change in mitochondrial function may play on total liver function in such areas as degradation of thyroid and other hormones, synthesis of plasma-binding proteins, lipid metabolism, and secondarily cardiac function remains to be explored.

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References


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