Serum Lactic Dehydrogenase Isoenzymes in "Myxoedema Heart Disease"

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The characteristic intracellular accumulations of homogeneous basophilic infiltrate, which are found in the myocardium of patients dying with chronic thyroid deficiency, are thought to represent a specific type of cardiomyopathy (Brewer, 1951; Means, DeGroot, and Stanbury, 1963). However, observations of this type have been infrequent, since histological material is rarely available from young patients with uncomplicated and untreated hypothyroidism. The hypothyroid patients who die are usually over 60 years of age and frequently have coexisting hypertension or coronary artery disease. In addition, treatment with thyroid hormone has often begun by the time of their death (Aber and Thompson, 1963; Means *et al.*, 1963).

Clinical recognition of myocardial damage resulting solely from chronic thyroid deficiency is difficult since heart failure rarely, if ever, occurs as a consequence of thyroid deficiency *per se* (Scheinberg *et al.*, 1950; Ellis *et al.*, 1952; Graettinger *et al.*, 1958; McBrien and Hindle, 1963; Aber and Thompson, 1964); and the characteristic electrocardiographic and radiological abnormalities of "myxoedema heart disease" can equally well be ascribed to the presence of pericardial fluid rather than to myocardial damage (Lerman, Clark, and Means, 1933; La Due, 1943; Kern *et al.*, 1949; Ellis *et al.*, 1952; Marks and Roof, 1953).

The results of the present investigation using isoenzyme studies, in addition to clinical, electrocardiographic, and radiographic observations, provide further evidence that a specific form of

myocardial damage occurs in patients with chronic thyroid deficiency and suggests a means of establishing such a diagnosis.

SUBJECTS AND METHODS

Forty-eight patients (10 men and 38 women) with chronic thyroid deficiency of varied aetiology have been studied (Table I). Thyroid deficiency was confirmed or established in 43 by radioactive iodine studies. Five patients with undoubted clinical myxoedema were not subjected to this test. The average age was 58 years (range 24–77 years).

A resting systolic blood pressure of 160 mm. Hg or more, or a diastolic pressure over 95 mm. Hg, was present in 19 patients before commencing treatment, and they were considered to be *hypertensive*.

Proven coronary artery disease, with at least one previous episode of myocardial infarction, existed in 10 patients:

Exertional chest pain was complained of by 6 patients—women between 30 and 61 years of age with no previous history of coronary disease. This pain had most of the features of angina of effort and had begun with their other symptoms of thyroid deficiency. One patient had established rheumatic mitral valve disease.

Electrocardiograms. Conventional 12-lead electrocardiograms were recorded before and during treatment until a euthyroid state had been established. Each patient was placed into one of two groups (A and B) according to their pretreatment cardiographic pattern (Aber and Thompson, 1963).

Group A. The electrocardiograms showed at least 3 of the 4 following features: (i) sinus bradycardia, (ii) generalized low voltage, (iii) prolongation of the P–R interval, and (iv) generalized flattening or inversion of the T waves.

Group B. The electrocardiogram was either normal, or it lacked the above criteria of "myxoedema heart disease"; or there was evidence of left ventricular hypertrophy or coronary artery disease.

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TABLE I

RELATION BETWEEN AETIOLOGY OF CHRONIC THYROID DEFICIENCY AND LDH ISOENZYME PATTERNS IN 48 PATIENTS

Aetiology of hypothyroidism	No. of patients	LDH isoenzyme pattern	
		Normal	Abnormal
Spontaneous	30	18	12
Hashimoto's disease	5	3	2
Methyl thiouracil therapy	6	5	1
Post-thyroidectomy	3	2	1
Hypopituitarism	3	3	—
Post- ¹³¹ I therapy	1	—	1
Total	48	31	17

There were 27 patients in Group A and 21 in Group B (Table II).

Heart Size. The cardiothoracic ratio (C/T) was used as an index of heart size. These measurements were made from conventional (6-foot) postero-anterior chest radiographs before and during the treatment period. The heart was considered to be of normal size when the C/T ratio was 50 per cent or less (30 patients), and enlarged when the C/T ratio was 51 per cent or more (18 patients) (Table II).

FIG. 1.—(A) Normal LDH isoenzyme pattern. (B) LDH isoenzyme pattern after myocardial infarction (3rd day) showing accentuation of LDH-1 and LDH-2 bands with staining of the adjacent gel. (C) and (D) Abnormal and normal LDH isoenzyme patterns in chronic thyroid deficiency. (C) shows a pattern similar to that of myocardial infarction.

TABLE II

RELATION BETWEEN PRETREATMENT LDH ISOENZYME PATTERNS AND ELECTROCARDIOGRAPHIC PATTERNS AND HEART SIZE IN 48 PATIENTS WITH HYPOTHYROIDISM

		LDH isoenzyme pattern	
		Normal	Abnormal
Electrocardiogram	Group A (27 patients)	15	12
	Group B (21 patients)	16	5 (0.05 < p < 0.1)
Heart size	C/T = 50% or less (30 patients)	17	13
	C/T = 51% or more (18 patients)	14	4 (0.05 < p < 0.1)

C/T = Cardiothoracic ratio.

Serum Lactic Dehydrogenase Isoenzymes. Visual demonstrations of the serum lactic dehydrogenase (LDH) isoenzyme distribution were made before and during hormone therapy using minor modifications of the vertical starch-gel electrophoretic method of Latner and Skillen (1961). Electrophoresis at 4°C. was maintained for 2½ hours, and, subsequently, enzyme activity was demonstrated on the surface of the sliced gel by staining in the dark for 1 hour at 37°C. The stained gel was then immediately dried and photographed.

Serum was kept at room temperature before electrophoresis, which was always carried out within 5 days of collection.

A normal serum LDH isoenzyme pattern is shown in Fig. 1A. The individual isoenzyme bands are labelled numerically according to their electrophoretic mobility. LDH-1, which is often difficult to demonstrate in serum from healthy subjects, travels furthest from the point of insertion (marked by the arrow) of the serum into the gel towards the anode. Its position is indicated by the numeral 1 and the dotted line in Fig. 1A. LDH-2, LDH-3, and LDH-4 are less mobile but also travel towards the anode, whereas LDH-5 migrates towards the cathode.

LDH-1 and LDH-2 occur in relatively high concentration in the human myocardium (Wróblewski and Gregory, 1961; Cohen, Djordjevic, and Ormiste, 1964). Following myocardial infarction, and other forms of myocardial damage (Blanchaer, 1961; Wróblewski and Gregory, 1961; Latner, 1962; Cohen *et al.*, 1964; Aber *et al.*, to be published), they are liberated into the circulation producing an isoenzyme pattern characterized by marked accentuation of both these isoenzyme bands and often staining of the adjacent gel (Fig. 1A).

Total serum lactic dehydrogenase was measured by the method of King (1959)—normal range = 225–540 units per cent.

Serum Vitamin B₁₂. Since (i) B₁₂ deficiency causes a serum LDH isoenzyme pattern very similar to that following myocardial infarction (Wróblewski and Gregory, 1961; Latner, 1962; Cohen *et al.*, 1964), and (ii) a small proportion of patients with thyroid deficiency also have B₁₂ depletion, all patients were

FIG. 2.—LDH isoenzyme patterns in 48 patients with chronic thyroid deficiency.

screened for evidence of B₁₂ deficiency, using the method of Matthews (1962).

Serum Transaminase (SGOT). Serum transaminase was estimated by the method of King (1960).

Circulating thyroid antibody studies were made as follows:

- Tanned red cell test*—by the method of Fulthorpe *et al.* (1961).
- Complement-fixation test*—by the technique of Forbes *et al.* (1962).
- Precipitin test*—by the method of Anderson *et al.* (1962).

RESULTS

LDH Isoenzyme Patterns. The serum of 17 of the 48 patients (35%) showed conspicuous accentuation of LDH-1 and LDH-2 bands, giving a very similar pattern to that following myocardial infarction (Fig. 1 and 2 and Table I). In the remaining 31 (65%) the isoenzyme pattern was apparently normal in so far as these two bands could be assessed (Fig. 1 and 2 and Table I). In contrast, the other three LDH isoenzyme bands (LDH-3, LDH-4, and LDH-5) varied in staining intensity, and formed no consistent distribution patterns.

Biochemical characterization of the isoenzymes in serum containing high concentrations of LDH-1 and LDH-2 showed that both these isoenzymes behaved in the same way as the two similar isoenzymes of myocardial infarction—they were both relatively heat stable at 60°C. and were differentially inhibited by high concentration of sodium lactate (0.72 M) in the incubating media.

On the other hand, LDH-3, LDH-4, and LDH-5 activity diminished much after heating the serum to 60°C., and was virtually unaffected by a high lactate concentration in the staining media.

No direct relation was found between these

isoenzyme patterns and either the severity of the clinical picture, the incidence of hypertension ($p > 0.4$), or the incidence of coronary artery disease ($p > 0.5$) before starting thyroid therapy. Similarly, no definite relation was observed between either isoenzyme pattern and the level of circulating thyroid antibodies or the cause of the thyroid deficiency. However, accentuated LDH-1 and LDH-2 bands have not yet been seen in hypopituitarism (3 patients) (Table I).

Most of the patients with "atypical" electrocardiographic patterns (Group B) or cardiac enlargement before commencing treatment had normal isoenzyme patterns (Table II).

Effects of Thyroid Therapy on the LDH Isoenzyme Patterns, the Electrocardiogram, and Heart Size.

LDH Isoenzymes. Gradual fading of the accentuated LDH-1 and LDH-2 bands was always noted after thyroid therapy (Fig. 3). The more rapidly a euthyroid state was re-established, the sooner these isoenzyme bands reverted to normal. However, fading of these bands was difficult to detect before the patients were receiving between 0.1 and 0.15 mg. L-thyroxine per day or an equivalent dose of diotroxin. In contrast, no change was observed in the isoenzyme pattern of those patients with normal pretreatment patterns (Fig. 4).

Electrocardiograms. Electrocardiographic improvement paralleled the resolution of the abnormal isoenzyme pattern in the 12 patients from Group A who showed accentuation of LDH-1 and LDH-2 bands before commencing treatment (Fig. 3 and Table II). However, similar electrocardiographic improvement occurred in the other 15 patients from Group A with normal pretreatment isoenzyme patterns (Fig. 4 and Table II).

FIG. 3.—A 40-year-old woman with spontaneous hypothyroidism—showing gradual resolution of abnormal LDH isoenzyme and electrocardiographic patterns with thyroid therapy:—

June 1, 1964: Pretreatment electrocardiogram and abnormal isoenzyme pattern.

July 13, 1964: Improvement of the electrocardiogram and isoenzyme pattern when the patient was receiving 0.15 mg. L-thyroxine per day.

September 7, 1964: Complete resolution of the electrocardiogram and isoenzyme pattern when patient became euthyroid on 0.3 mg. L-thyroxine per day.

Of the 5 patients from Group B who had abnormal isoenzyme patterns before treatment, 3 showed no electrocardiographic improvement with thyroid hormone; whereas the electrocardiograms of the other 2 showed striking improvement by the time they were euthyroid (Fig. 5).

Heart Size. In 16 of the 17 patients with abnormal isoenzyme patterns, a reduction in heart size of at least 10 per cent of the initial C/T ratio had occurred by the time they became euthyroid. This decrease in heart size occurred whether the heart was enlarged (4 patients), or within normal limits

FIG. 4.—A 50-year-old woman with spontaneous hypothyroidism—showing a normal pretreatment LDH isoenzyme pattern and an electrocardiogram characteristic of "myxoedema heart disease" (April 29, 1964):

June 12, 1964: Improvement in the electrocardiographic pattern but no change in the isoenzyme pattern when the patient was receiving 0.15 mg. L-thyroxine per day.

September 2, 1964: Complete resolution of the cardiogram but still no change in the isoenzyme pattern when the patient was euthyroid on 0.3 mg. L-thyroxine per daily.

FIG. 5.—A 62-year-old man with spontaneous hypothyroidism with an abnormal LDH isoenzyme pattern and "atypical" cardiogram (Group B) before starting thyroid therapy (October 8, 1964). Striking electrocardiographic improvement along with resolution of the isoenzyme pattern when he became euthyroid (January 29, 1965).

(12 patients), before treatment was started (Fig. 6A and B). However, 3 of the 4 patients with an enlarged heart and an abnormal isoenzyme pattern did not have hearts of normal size by the time they were euthyroid. Two of these patients had moderately severe hypertension before and after treatment, whereas the third had severe mitral valve disease. Only one patient with abnormal isoenzymes failed to show a 10 per cent reduction in heart size when euthyroid.

In contrast, none of the 31 patients with normal isoenzyme patterns demonstrated a reduction in heart size with thyroid treatment (Fig. 7A and B).

Of the 31 patients with normal isoenzymes, 14 had enlarged hearts. All were over 60 years of age and had either coronary artery disease or hypertension;

and their electrocardiograms were not characteristic of "myxædema heart disease" (Group B) (Table II).

Coronary Artery Disease. Of the 10 patients with proven coronary artery disease, 3 had abnormal isoenzyme patterns which gradually resolved with cautious thyroid therapy. One of the 7 patients with coronary artery disease and initially normal isoenzymes had a myocardial infarct 6 weeks before beginning thyroid treatment: 6 weeks later, whilst receiving 0.1 mg. L-thyroxine daily, she had a further infarct. Retrospective examination of her serial isoenzyme patterns revealed that an abnormal pattern (with accentuation of LDH-1 and LDH-2 bands) had appeared 5 weeks before this second infarct (Fig. 8). Subsequently, 2 other patients

FIG. 6.—(A) A 52-year-old woman with Hashimoto's disease—with a normal-sized heart and an abnormal LDH isoenzyme pattern before thyroid therapy (April 25, 1964). Complete resolution of the isoenzyme pattern with 15 per cent reduction in heart size when she became euthyroid (August 22, 1964). (B) A 62-year-old woman with spontaneous hypothyroidism—with an enlarged heart and abnormal LDH isoenzyme pattern before thyroid therapy (January 7, 1965). Complete resolution of the isoenzyme pattern with 16 per cent reduction in heart size when she became euthyroid (March 23, 1965).

FIG. 7.—(A) A 36-year-old woman with spontaneous hypothyroidism—with a normal-sized heart and normal LDH isoenzyme pattern before thyroid therapy (April 23, 1964). The heart size and isoenzyme pattern remained unchanged when she became euthyroid (October 1, 1964). (B) A 64-year-old woman with spontaneous hypothyroidism—with an enlarged heart and a normal LDH isoenzyme pattern before thyroid therapy (September 17, 1964). The heart size and isoenzyme pattern remained unchanged when she became euthyroid (March 18, 1965).

FIG. 8.—A 58-year-old woman with spontaneous hypothyroidism and proven coronary artery disease. January 19, 1965: The cardiogram and normal LDH isoenzyme pattern 6 weeks after her first myocardial infarction—before starting thyroid therapy. January 26, 1965: After 1 week's treatment with 0.05 mg. L-thyroxine daily; the cardiogram remained the same whereas her LDH isoenzyme pattern showed LHD-1 and LHD-2 bands with staining of the adjacent gel. March 9, 1965: Six weeks after starting treatment there was clinical, electrocardiographic, and LDH isoenzyme evidence of a further myocardial infarct while on 0.1 mg. L-thyroxine daily.

with coronary artery disease and normal isoenzyme patterns complained of chest pain while receiving thyroid hormone. However, their isoenzyme patterns remained normal, as did their electrocardiograms and SGOT levels. Therefore, it was considered safe to continue treatment. Six months later both patients were euthyroid and had experienced no deterioration in cardiac reserve, nor had they had further chest pain.

Exertional Chest Pain. Of the 6 patients who complained of exertional chest pain, 5 had abnormal isoenzyme patterns and all had electrocardiograms that were characteristic of "myxoedema heart disease" (Group A). Their pain always disappeared as they became euthyroid along with return of their isoenzyme patterns and electrocardiograms to normal. Of these 5 patients, 4 have had no further chest pain. One, a 56-year-old woman, died from peritonitis 1 month after becoming euthyroid. Subsequent post-mortem coronary angiograms (Fig. 9) and histological examination of the heart revealed no evidence of coronary artery disease. The sixth patient with "exertional chest pain", but no previous history of coronary artery disease, had a normal isoenzyme pattern before treatment. The

pattern remained normal until she was euthyroid though she experienced several episodes of resting chest pain during the treatment period. Subsequently, her pain disappeared completely (Fig. 4).

Total LDH. The total serum LDH was estimated in the first 20 patients of this series. In 17, the level was within the normal range (mean = 412 units %; range 270–525). In the remaining 3 (all patients with abnormal isoenzyme patterns), it was raised (620, 680, and 740 units %). Since 7 of the patients with isoenzyme patterns showing marked accentuation of LDH-1 and LDH-2 bands had normal total LDH levels, no further measurements of total LDH were made.

Serum Transaminase (SGOT). Minimal increases in the SGOT were observed in 3 patients with abnormal isoenzyme patterns before treatment (95, 110, and 115 units %). In the remaining 14 it was within normal limits (mean = 70 units %, range 45–85 units %).

Serum Vitamin B₁₂. All patients in the series had normal serum B₁₂ levels (mean = 230 $\mu\mu$ g./100 ml.; range 140–750 $\mu\mu$ g./100 ml.).

FIG. 9.—Post-mortem coronary angiograms on a 56-year-old woman who 3 months previously had had spontaneous hypothyroidism. Before treatment she had an abnormal LDH isoenzyme pattern (below angiograms), yet necropsy revealed a normal coronary arterial tree.

DISCUSSION

Accentuation of the first two LDH isoenzyme bands (LDH-1 and LDH-2) had been noted in hæmolytic anæmia, acute pancreatitis, renal infarction, and occasionally in hæmorrhagic shock (Vesell, 1961; Wróblewski and Gregory, 1961; Latner, 1962, 1964; Cohen *et al.*, 1964). In the absence of these conditions, differential accentuation of the LDH-1 and LDH-2 bands has only been described in association with certain forms of myocardial damage: myocardial infarction; acute rheumatic myocarditis; following cardiac massage; and in hypothermic myocardial damage (Wróblewski and Gregory, 1961; Latner 1962, 1964; Cohen *et al.*, 1964; Aber *et al.*, to be published). Therefore, the discovery in some patients with hypothyroidism of a LDH isoenzyme pattern with electrophoretic and biochemical characteristics similar to those of myocardial infarction, is suggestive of the presence of myocardial damage in these patients (Wróblewski and Gregory, 1961; Latner and Skillen, 1963; Plummer and Wilkinson, 1963; Brody, 1964). Furthermore, since there was no relation between this abnormal isoenzyme pattern and either the presence of coronary artery disease or hypertension,

it is likely that these findings indicate the presence of myocardial damage due to thyroid deficiency *per se*. The reduction in heart size in 16 of the 17 patients with abnormal LDH isoenzymes following hormone treatment also suggests a specific myocardial lesion in hypothyroidism, since both improvement in myocardial function and decrease in myocardial compliance are as likely to be responsible for these radiological changes as reabsorption of pericardial fluid. The absence of a significant reduction in heart size in those patients who had normal (LDH) isoenzyme patterns and enlarged hearts before treatment can be explained by their age and associated coronary artery disease and hypertension. Furthermore, their electrocardiograms were usually not characteristic of "myxædema heart disease"—Group B (Aber and Thompson, 1963). On the other hand, when there was no cardiac enlargement and a normal isoenzyme pattern, significant myocardial damage and pericardial effusions were less likely, and consequently reduction in heart size would not be expected.

Serial LDH isoenzyme studies can help with the management of those patients with chronic thyroid deficiency who also have established coronary artery

disease—a difficult and at times worrying group of patients. Progressive fading of accentuated LDH-1 and LDH-2 bands, or alternatively, persistence of a normal isoenzyme pattern despite complaints of “chest pain” are encouraging and will allow cautious continuation of the treatment régime. However, the development of accentuated LDH-1 and LDH-2 bands in the serum of patients who previously had normal isoenzyme patterns, or subsequent reappearance or immediate intensification of these two bands in patients with abnormal patterns before treatment, may well herald clinical coronary insufficiency or myocardial infarction.

The hormone-induced electrocardiographic improvement in 2 of the 5 patients in Group B with abnormal isoenzymes was probably the result of resolution of myocardial damage due to chronic thyroid deficiency. Failure to observe any change in the electrocardiograms of the other 3 patients, 2 with severe hypertension and 1 with advanced mitral valve disease, probably means that their electrocardiographic patterns were determined predominantly by these associated conditions.

The significance of “exertional chest pain” in 6 patients with no previous history suggesting coronary artery disease is not clear. However, 5 of them had abnormal isoenzyme patterns, and as they became euthyroid their chest pain disappeared and their isoenzyme and electrocardiographic patterns and heart size reverted to normal. The possibility of a thyroid deficiency cardio-skeletal myopathy is suggested by the occurrence of both cardiac and skeletal muscle lesions and the frequent presence of “pseudo-claudication” calf pain in chronic thyroid deficiency (Brewer, 1951; Adams, Denny-Brown, and Pearson, 1962; Means *et al.*, 1963). Whether the chest pain arises in the chest wall muscles or in the myocardium is not known.

Total serum LDH estimations were unhelpful in studying the present problem. However, this is not surprising since the total serum LDH level varies very much in health and can remain within normal limits even though the LDH isoenzyme pattern shows evidence of myocardial damage (Latner and Skillen, 1961; Cohen *et al.*, 1964). Similarly, the serum transaminase failed to identify the presence of myocardial damage in most of those patients with abnormal LDH isoenzymes. Perhaps the thyroid-deficient myocardial lesion develops too slowly to allow a detectable increase in the serum transaminase level.

Unfortunately, the present observations do not indicate the mode of development of myocardial damage in chronic thyroid deficiency; nor do they offer a satisfactory explanation for the characteristic electrocardiographic pattern of “myxœdema heart

disease”. Detailed histological and histochemical animal studies are in progress in an attempt to answer some of these outstanding problems.

SUMMARY

Using vertical starch-gel electrophoresis, serum lactic dehydrogenase isoenzyme studies have been made on 48 patients with hypothyroidism. In 17 (35%), the isoenzyme distribution pattern resembled that of acute myocardial infarction with differential accentuation of LDH-1 and LDH-2 isoenzyme activity. It is suggested that this isoenzyme evidence of myocardial damage represents a specific form of cardiomyopathy resulting from chronic thyroid deficiency.

Re-establishment of a euthyroid status with hormone replacement therapy was accompanied by gradual disappearance of the “heart” isoenzyme distribution, electrocardiographic improvement, and reduction in heart size. In contrast, no reduction in heart size was observed in the other 31 patients in the series who had normal LDH isoenzyme patterns before starting thyroid therapy.

Serial LDH isoenzyme studies provided considerable help in the management of those patients with coronary artery disease and hypothyroidism in whom too rapid or excessive treatment may provoke myocardial infarction.

Evidence is also presented to show that some patients with chronic thyroid deficiency might have a cardio-skeletal myopathy manifest clinically as “exertional chest pain” and usually thought to be due to associated coronary artery disease. However, this type of pain disappears with adequate hormone therapy.

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