SERUM ENZYME PATTERNS IN ACUTE ISCHEMIC HEART DISEASE WITH SPECIAL REFERENCE TO LDH ISoenZYMES IN INTERMEDIATE TYPES OF ISCHEMIC HEART DISEASE, FRESH MYOCARDIAL INFARCTION, AND CARDIOGENIC SHOCK

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Lactic Dehydrogenase (LDH) was first extracted, purified and crystallized from four bullocks' hearts by Straub in 1940. Then the crystalline lactic dehydrogenase of Straub was separated into two catalytically active fractions. Thereafter, LDH was further separated into five fractions by electrophoresis by many investigators, and it has been reported that the LDH of human and animal tissues and body fluids consists of at least five different fractions called LDH isoenzymes which can be separated by electrophoresis.

Appella and Markert reported that each fraction (isoenzyme) is a tetramer which may be split into four monomers, and the five LDH isoenzymes are formed from two different types of monomer, M and H. The sub-unit structure of LDH has been confirmed by many other investigators.

Clinical examinations, first reported by Hill & Levi in 1954, showed that serum LDH activity was increased in patients with malignant tumors. The determination of SLDH activity was adopted as a method of diagnosis of malignant tumors, but it was soon noted that it was increased in a wide variety of pathological conditions, such as cardiac, hepatic, hematologic, muscular, renal and other diseases, as well as in malignant tumors.

In 1957 Wieland and Pfeiderer found that in most organs all five fractions of LDH activity were present.

In 1960 and 1961, Wróblewski et al. examined the electrophoretic pattern of LDH isoenzymes in the sera and various organs of patients and found similar electrophoretic patterns in the serum and damaged organs.

In 1964 Cohen et al. described the great diagnostic value of the electrophoretic pattern of SLDH isoenzymes in patients with cardiovascular diseases.

Recently, serum LDH isoenzymes have been studied in many diseases, and the electrophoretic pattern of SLDH isoenzymes has been found to be a very useful diagnostic tool.

The author has analyzed the electrophoretic patterns of SLDH isoenzymes in more than 3,000 specimens obtained from patients with various diseases.

The present paper describes the serial changes of various serum enzymes in acute ischemic heart disease, with special reference to the electrophoretic pattern of LDH isoenzymes.

MATERIALS AND METHODS

Serum was obtained from 50 patients with ischemic heart disease accompanied by chest pain. Generally, ischemic heart disease with chest pain can be further classified as angina of effort, intermediate types of ischemic heart disease and myocardial infarction, but the distinction among

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these diseases remains unclear.

In the present study, ischemic heart disease was subdivided into three groups: angina of effort, intermediate types of ischemic heart disease, and fresh myocardial infarction; in addition, the WHO classification of clinical symptoms and electrocardiographic findings was used. The number of patients in each group was as follows: 1) Fresh myocardial infarction, 17 cases; 2) angina of effort, 13 cases; and 3) intermediate types, 20 cases. Sera from 50 normal subjects were analyzed as controls.

Blood was obtained by venepuncture usually from the cubital vein and allowed to clot at room temperature for about one hour. The clotted blood was centrifuged at 3,000 r.p.m. for about ten minutes.

SGOT and SGPT activities were determined by the method of Reitman and Frankel21 and SCPK activity by the method of Nielsen-Ludvigsen using “CPK STAT-PACK” made by CALBIOCHEM. SLDH activity was determined by the method of Caubaud-Wróblewski22.

Numerous modification of electrophoretic and chromatographic techniques for the separation of LDH isoenzymes have been described. In the field of clinical biochemistry, electrophoresis, a convenient method for the separation of LDH isoenzymes, has been used extensively. In recent years, a number of alternative methods employing a variety of supporting media, e.g. starch granules, cellulose acetate, starch gel, agar gel, polyacrylamide, etc., have been introduced23-29.

The electrophoretic pattern of SLDH isoenzymes was analyzed by means of “agar-agarose”30 gel electrophoresis by a modification of the method of Wieme23 and Yoshida29 a technique employing microscope slides to support the agar gel, and the fractions were designated LD5, LD4, LD3, LD2, and LD1, seriatim from the anode side to the cathode side corresponding to the report of Wróblewski.

AGAR-AGAROSE gel electrophoresis
Buffer stock solution.

a) Sodium veronalate-HCl buffer: pH 8.4, μ = 0.04, sodium veronalate 8.5 g., hydrochloric acid (N) about 11.5 ml.,aq. dist. ad. 1,000 ml.

b) Tris(hydroxymethyl)aminomethane-HCl buffer: pH 7.4, Tris(hydroxymethyl)aminomethane 7.2 g., hydrochloric acid (N) about 50 ml.,aq. dist. ad. 1,000 ml.

Preparation of the agar-agarose gel plates
Heat the veronalate-HCl buffer solution in a boiling water bath. Add the agar-agarose powder (Difco Special Agar-Noble, 0.7 g., and Behring Agarose, 0.1 g. for 100 ml. buffer) and heat the solution further until entirely clear. Pour 50 ml. of clear and warm agar-agarose solution into a petri dish to cover the bottom entirely. When a perfectly horizontal surface has been obtained, and the agar-agarose has solidified, place the four glass slides carefully on this surface, then cover with 50 ml. more of the agar-agarose gel solution.

Application of the sample.
Cut out a glass slide covered with the agar-agarose layer, and put two small strips (1.5 x 5 mm.) of filterpaper 3 mm. to the left of the center of the slide. Introduce 4 to 50 μl. of serum solution depending on SLDH activity, onto the filterpaper by micropipette.

Electrophoretic run.
Place the slides in the electrophoretic box and fill the buffer vessels with the sodium veronalate-HCl buffer and the central tank with petroleum ether (this box was made by Fujiriken Co. Ltd. Japan.). Electrophoresis proceeds under the control of a constant current, 60 mA. for 90 min.(4 slides).

Staining.
The SLDH isoenzymes are detected by incubating the agar-agarose slides in the dark for one hour at 37.0°C. using the following staining solution(this solution should be prepared just before use).

Tris-HCl buffer pH 7.4, 0.05 M. 56 ml.
KC N 0.06 M. 5 ml.
Sodium lactate 2 M. 4 ml.
Nicotine amide adenine
dinucleotide(NAD) 40 mg.
Nitroblue tetrazolium(NBT) 30 mg.
Phenazine methosulphate(PMS)
20 mg./dl. 5 ml.

Fixation.
The agar-agarose plates are fixed for 20 minutes in about 100 ml. of a 14:5:1 by volume solution of water, methanol, and acetic acid, and then immersed in a fresh solution for about 6 hours.

Drying.
Cover the agar-agarose plates with a sheet of filterpaper and dry at 37.0°C, rinse well with 5 % acetic acid and water, and remove the filterpaper. Quantitative estimation.
The five purple bands of SLDH isoenzymes are scanned in a densitometer at 570 mp to obtain quantitative values as percentages of each

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fraction.

RESULTS

I) Controls.
The sera of 50 healthy persons were found to have a characteristic distribution of SLDH isoenzymes, that is, LD1<LD2<LD3<LD5<LD4, as shown in Fig. 1. The pattern with an LD5/LD4 ratio of more than 0.81 was referred to as H-type, and that with more than 7.2% LD1, as M-type. Patterns within the normal range were termed N-type. The activities of various enzymes in the sera of 50 healthy persons are summarized and tabulated in Table 1.

II) Acute ischemic heart disease with chest pain.
1) Fresh myocardial infarction:
In the group of patients with fresh myocardial infarction (17 cases), SLDH activity and the LD5/LD4 ratio began to increase 2–4 hours after the attack to reach peak values in about 2 days (Fig. 2). Thereafter, SLDH activity gradually decreased to normal in about 2 weeks, while the LD5/LD4 ratio became normal in 3 weeks on the

![Fig.1. 50 normal subjects.](image)

**TABLE 1 MEAN VALUES OF ACTIVITIES OF VARIOUS SERUM ENZYMES IN 50 HEALTHY PERSONS**

<table>
<thead>
<tr>
<th>Serum enzyme</th>
<th>Mean</th>
<th>S. D.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOT</td>
<td>16.2 (R.F unit)</td>
<td>±5.8 (R.F unit)</td>
<td>4.6–27.9 (R.F unit)</td>
</tr>
<tr>
<td>GPT</td>
<td>12.7 (R.F unit)</td>
<td>±7.4 (R.F unit)</td>
<td>0–27.5 (R.F unit)</td>
</tr>
<tr>
<td>CPK</td>
<td>23.9 (mU/ml)</td>
<td>±9.0 (mU/ml)</td>
<td>5.9–42.0 (mU/ml)</td>
</tr>
<tr>
<td>LDH</td>
<td>234.0 (W unit)</td>
<td>±37.7 (W unit)</td>
<td>158.6–309.4 (W unit)</td>
</tr>
<tr>
<td>LD5</td>
<td>23.1 (%)</td>
<td>±5.9 (%)</td>
<td>11.4–34.7 (%)</td>
</tr>
<tr>
<td>LD4</td>
<td>48.1 (%)</td>
<td>±6.7 (%)</td>
<td>34.6–61.6 (%)</td>
</tr>
<tr>
<td>LD3</td>
<td>20.9 (%)</td>
<td>±4.9 (%)</td>
<td>11.6–30.6 (%)</td>
</tr>
<tr>
<td>LD2</td>
<td>4.9 (%)</td>
<td>±2.5 (%)</td>
<td>0–9.8 (%)</td>
</tr>
<tr>
<td>LD1</td>
<td>3.1 (%)</td>
<td>±2.1 (%)</td>
<td>0–7.3 (%)</td>
</tr>
<tr>
<td>LD5/LD4</td>
<td>0.50</td>
<td>±0.16</td>
<td>0.18–0.81</td>
</tr>
</tbody>
</table>

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average as seen in Fig. 2. SGOT and SCPK activities began to increase about 4 hours after the attack, reached their peaks in about 12 hours and returned to normal in about 7 days.

The serial changes of various serum enzymes in a typical case of fresh myocardial infarction were as follows:

CASE.—K.S., a 59-year-old male had a history of hypertension since 1963. On Jan. 27, 1969, he was hospitalized because of precordial pain.

On physical examination, the patient appeared to be in pain, his pulse was 90 per minute and his blood pressure 150/90 mmHg. The heart was not enlarged, and sounds were clear. There were no abnormal findings in lungs, abdomen, or extremities. The first electrocardiogram taken during a period of increasing chest pain, showed ST elevation, and Q waves in leads II, III, and aVF, with reciprocal ST depression in leads I, aVL, and V3 through V5, as shown in Fig. 3-A. This case was diagnosed as fresh inferior wall myocardial infarction. Chest pain was relieved by

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medical treatment. The patient again suffered chest pain for about 10 minutes on the 4th and 7th days after admission. Electrocardiograms taken during these anginal attacks, showed ST depression in leads V4 through V6.

Serial studies of serum enzymes in this case showed a marked increase of SLDH, SGOT, SCPK and the LD5/LD4 ratio after the attack with a gradual decrease of SGOT and SCPK, but SLDH and the LD5/LD4 ratio rose again after the anginal attacks on the 4th and 7th days. SGOT and SCPK values became normal within 10 days, and SLDH and the LD5/LD4 ratio within 25 days after the attack (Fig.3-B).

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2) Angina of effort:

In the group of patients with angina of effort (13 cases), the various serum enzyme activities remained in the normal range.

A typical case of angina of effort:

M.K., a 41-year-old male had a history of hypertension for the past 6 years, and had begun to feel chest pain on effort, which was relieved by resting for about 10 minutes. The frequency of his chest pain on effort increased during the month before admission on Jan. 16, 1970. There were no abnormal physical findings, but the electrocardiogram taken during chest pain after exercise showed ST depression in leads V4 through V6, and low amplitude T waves. After the anginal attack, the electrocardiogram returned to normal (Fig.4-A). The various serum enzymes remained in the normal range (Fig.4-B).

3) Intermediate types of ischemic heart disease:

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In the group of patients with intermediate types of ischemic heart disease, there were four types of electrophoretic patterns of SLDH isoenzymes: infarction type, dissociation type, abortive type, and normal type (Fig. 5).

a) Infarction type (five cases).

Serial changes of the various serum enzymes (SLDH, SGOT, and SCPK) and the electrophoretic pattern of SLDH isoenzymes (i.e., LDS/LD4 ratio) were similar to those in typical fresh myocardial infarction, as seen in Fig. 5, but serial electrocardiograms showed no characteristic evidence of myocardial infarction.

Two typical cases of this infarction type are described below.

CASE-T.S., a 37-year-old male had a history
of hypertension and fatty liver in 1966. The patient had a sudden chest pain at about 2:00 p.m., June 22, 1969. On physical examination, the patient was fat, and appeared to be in pain. His pulse was 100 per minute, and his blood pressure was 160/100 mmHg. The heart was not enlarged, and sounds were clear. The liver edge was felt about 3 cm. below the right costal margin at the midclavicular line. There were no other abnormal findings in lungs, abdomen, or extremities. Fig.6-a, b shows the electrocardiograms taken in 1966 and during the anginal attack after admission (22/6/69). The latter shows slight sinus tachycardia but no other abnormal findings. Two days after admission, the patient again had acute precordial pain. The electrocardiogram taken during this second attack also shows only sinus tachycardia and low amplitude T waves (Fig.6-c). The vectorcardiogram taken after the attack also shows no characteristic ischemic changes (Fig.7).

As seen in Fig.8, SCPK, SGOT, SLDH activities and the LD5/LD4 ratio increased after the attack and then gradually returned to normal. SCPK returned to normal most rapidly. Both SLDH and the LD5/LD4 ratio became normal about 10 to 14 days after the attack. The return to normal of SGOT was later than in other cases, presumably because of the fatty liver.

CASE-M.K., a 62-year-old female had a 6 year history of hypertension and coronary insufficiency. She had complained of retrosternal “pressure” early in the morning on May 27, 1969, lasting for about one hour. On physical examination, the patient appeared to be in pain, but no other abnormal findings were noted. The first electrocardiogram was taken in 1963 (Fig.9-a). The second electrocardiogram, taken

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during her anginal attack just after admission (Fig. 9-b), showed slight ST depression in leads 1, aVL, and V3 through V6, and abnormally low T waves in leads V1 through V6. In the electrocardiogram taken the day after the anginal attack, the T wave was distinctly inverted in lead aVL (Fig. 9-c). The ST depression and inverted T wave had become isoelectric in all leads by June 11, 1969, so there were no typical electrocardiographic signs of myocardial infarction. SGOT and SCPK activities rose within 4 to 12 hours after the anginal attack. The elevated SGOT level persisted for about 5 days, whereas the SCPK remained high for 9 days. The SLDH activity gradually decreased to normal in about 2 weeks, while the LD5/LD4 ratio became normal after more than 15 days, as seen in Fig. 10. The serial changes of these serum enzymes resembled those in typical fresh myocardial infarction.

b) Dissociation type (five cases).

The SLDH activity remained within the normal range, but the serial changes in the LD5/LD4 ratio were very close to those in fresh myocardial infarction, while SGOT and SCPK activities displayed a transient slight increase, as shown in Fig. 5.

A typical case of the dissociation type follows.

CASE. M.Y., a 59-year-old male had a history of myocardial infarction in 1963, and subarachnoid hemorrhage in 1966 (Fig. 11-a). On Nov. 4, 1969, he had acute precordial pain and dyspnea lasting for about 40 minutes and was hospitalized. The electrocardiogram taken during his anginal attack showed marked ST elevation in

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Fig. 7. Vectorcardiogram taken after attack in case T. S. (37 year-old male). (Infarction type).

Fig. 8. Serial changes of various serum enzymes, BSR, and WBCC in case T. S. (Infarction type).
leads I, aVL, and V2 through V6, and reciprocal ST depression in leads III, and a VF, as shown in Fig. 11-b. After the anginal attack, the ST changes became isoelectric in all leads, and the T waves were distinctly inverted in leads I, aVL, and V4 through V6 (Fig. 11-c). These T waves attained their greatest degree of inversion gradually, until two days after the anginal attack when the electrocardiogram showed giant negative T waves. Then the T waves gradually became normal, as shown in Fig. 11-d. On the 15th day after the anginal attack (Fig. 11-e), the T waves again became negative after mild exercise, although the patient did not complain of chest pain. A double Master's "2-step" test on May 19, 1970, was negative. As seen in Fig. 12, the values of SGOT, SCPK, and white blood cell counts were elevated slightly and transiently after the anginal attack. The serial changes of the LD5/LD4 ratio were very similar to those seen after myocardial infarction, although the increase of SLDH activity remained within the normal range. On the 16th day, the LD5/LD4 ratio again increased after exercise and then returned to normal within 3 weeks.

c) Abortive type (five cases).
The activities of the various serum enzymes remained almost within the normal range. The
LD5/LD4 ratio, however, showed a transient increase, as seen in Fig.5.

A typical case of the abortive type follows.

CASE.-S.K., a 41-year-old female had a history of pulmonary tuberculosis in 1960. She had been under medical treatment for coronary insufficiency for about four months prior to this hospitalization. The patient awoke feeling retrosternal “pressure” lasting for about 40 minutes at midnight on Oct. 31, 1970. The electrocardiogram taken during the anginal attack showed sinus tachycardia, low amplitude T waves in leads I and aVL, and marked ST depression and deeply inverted T waves in leads V2 through V5, when compared with the electrocardiogram taken four months prior to this hospitalization. After the attack, the ST segment reverted to its pre-attack level, but the low amplitude T waves in leads I and aVL, and the inverted T waves (coronary T waves) in V2 through V5 remained, as seen in Fig.13-a.

The serial changes of the various serum enzymes in this case are shown in Fig.13-b. The LD5/LD4 ratio increased transiently, although the activities of SLDH, SGOT, SGPT remained within the normal range.

d) Normal type (five cases).

The total SLDH activity and LD5/LD4 ratio did not increase during or after the anginal attack as seen in Fig.5. A slight increase of SCPK activity, however, was observed in two of the five patients. There was no abnormal change in the activities of the other serum enzymes. Three of the patients with this normal type, Prinzmetal’s "variant form" of angina pectoris are described here.1

CASE.-K.Y., a 57-year-old male had a history of hypertension since 1960 and had been under medical treatment for hypertension for one month prior to admission on Aug. 4, 1969. His illness began some 7 days before admission when he complained of sudden chest pains relieved by resting for about 25 minutes. At the time of hospitalization, the patient appeared to be in pain, but there were no abnormal findings except for hypertension (160/100 mmHg) and abnormal electrocardiogram. As seen in Fig.14, the electrocardiogram taken during the anginal attack in the hospital, showed marked ST elevation in leads V1 through V4, which returned to normal in about 30 minutes. This patient had similar daily Prinzmetal’s anginal attack at rest.

As seen in Fig.14, the serum enzymes remained normal during and after the anginal attack.

CASE.-K.A., a 48-year-old male had a history of cerebral hemorrhage in 1970. Ten days prior to his admission on March 10, 1971, he started to have sudden chest pains almost daily lasting 30 to 60 sec. On physical examination, there were no abnormal findings except for hypertension (160/100 mmHg). The electrocardiogram taken when there were no symptoms showed small q waves in leads II, III, and aVF; a low amplitude T wave in lead I; and ST depression and inverted T wave in lead aVL. Fig. 15 shows electrocardiograms recorded cotinuously by monitor ECU-Model 50 before, during and after an attack; first a low T wave, then gradually ST elevation, and following the decrease of ST elevation, the T wave became sharp, and the ST segment fell below the isoelectric line. As the chest pain abated, the T waves and ST segments returned to normal. Although the electrocardiogram showed dramatic changes during the anginal attack, there were no abnormal changes of serum enzymes except for a transient increase of SCPK activity after the attack (Fig.16).

CASE.-S.K., a 68-year-old male had a history of pulmonary tuberculosis since 1957 and surgery for gastric ulcer in 1965. On Feb. 15, 1969, he suddenly developed right hemiplegia and aphasia but remained conscious. The next day, the patient was hospitalized. On physical examination, he was well-developed, and well-nourished, his pulse was 70 per minute and regular, blood pressure 140/90 mmHg. His pupils reacted normally to light. The heart was not enlarged, and sounds were clear. Right patellar and achilles

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Fig.11. Serial changes of electrocardiogram in case M. Y. (Dissociation type).

a) Electrocardiogram taken about 3 years before the attack (22/8/69).
b) Electrocardiogram taken during anginal attack (5/11/69).
c)–h) Serial changes of electrocardiograms after attack.
reflexes were increased, and the Babinski sign was positive on the right.

A slight, transient increase of SLDH and SGOT activities due to the cerebral injury were observed on the first day after admission. For the first 3 days after admission, the patient had no complaints, but on the fourth day at 7:30 p.m., he suffered from chest pain. The electrocardiogram taken during the anginal attack, showed ventricular extrasystoles in lead I, ST elevation in leads II, III, and aVF, and ventricular tachycardia (Fig.17). After the chest pain subsided, the electrocardiogram returned to normal. As seen in Fig.17, during and after the anginal attack, the various serum enzymes remained within the normal range except for a transient, slight increase of SCPK activity. This patient died suddenly with arrhythmia.

Although the above three patients showed marked changes in the electrocardiogram during the attack, the various serum enzymes remained within the normal range except for a slight, transient increase of SCPK activity after the anginal attack.

III) Significance of M-type SLDH isoenzymes in acute ischemic heart disease.

Some patients with ischemic heart disease have been reported who develop M-type SLDH isoenzymes, but their origin and clinical significance are still obscure.

The author attempted to correlate the autopsy and clinical findings in the two patients who died of fresh myocardial infarction (Fig.18).

CASE. K.W., a 46-year-old male had been under medical treatment for hypertension since 1966. The patient complained of severe chest pain at 7:00 p.m. on Oct. 29, 1969, and was soon hospitalized, because of persisting chest pain. On physical examination, the patient was thin and appeared to be in pain. Consciousness was preserved. His pulse was 60 per minute and regular; his blood pressure was 60/55 mmHg, and his temperature was 35.0°C. The heart was not enlarged, sounds were normal, and no murmurs were detected in any area. The first electrocardiogram taken during a period of increasing pain showed signs of a wide fresh anterior myocardial infarction. The second electrocardiogram taken about 2 and a half hours after the attack showed nodal rhythm, and the

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Fig.12. Serial changes of various serum enzymes, BSR, and WBCC in case Y. M. (Dissociation type).

Fig.13. Serial changes of electrocardiograms and various serum enzymes in case S. K. (Abortive type).
third electrocardiogram taken 6 hours after the attack, showed many ventricular extrasystoles (Fig.19).

About 10 hours after the attack, SLDH activity was 1,430 W·U, the LD5/LD4 ratio was 1.45, and LD1 activity was 27.0%, so the pattern of SLDH isoenzymes in this case was of the "H+M" type, as seen in Fig.20. There was an increase of SGOT (91 R-FU), SCPK (57 mL/U), and SGPT (51 R-FU). This patient did not recover from severe cardiogenic shock and died about 15 hours after the onset of pain.

Postmortem examination showed that the left coronary artery was completely occluded by fresh thrombosis and recent myocardial necrosis involving the anterior and lateral walls of the heart with severe bleeding, and in those areas were noted three areas of torn heart muscle. Microscopic examination of the liver showed congestion and central necrosis presumably due to cardiogenic shock (Fig.21-B).

CASE-T.K., a 77-year-old male complained of sudden severe chest pain at 2:00 p.m. on July 3, 1969. The pain continued until he was hospitalized at 4:20 p.m. the same day. On physical examination, the patient was well-nourished and well-developed, but appeared to be in pain. Consciousness was preserved. His blood pressure was 110/70 mmHg; his pulse 35 per minute, and irregular. Heart sounds were normal, and no murmurs were detected in any area. The electrocardiogram taken during a period of increasing pain, showed sinus bradycardia and ST elevation in leads II, III, aVF, and VI through V3. The second and the third electrocardiograms taken 3 hours and 7.5 hours after the onset of pain showed gradual normalization of ST segments except in leads V1 through V3, as seen in Fig.22. About 4 hours after the onset of pain, the SLDH activity was 510 W·U., the LDS/LD4 ratio 0.62,
and the LD1 activity was 28.1%. Thus, the electrophoretic pattern of SLDH isoenzymes in this case was definitely “M-type” as seen in Fig. 23. There was also a slight increase of SGOT (91 R-FU), SGPT (51 R-FU), and SCPK (45 mU/L). The patient did not recover from cardiogenic shock and died about 10 hours after the onset of the attack. Postmortem examination: Fig. 24 shows a postmortem coronary angiogram in which both the right and the left coronary arteries show stenosis. The main trunks and branches of both coronary arteries, especially the right were occluded by dense old fibrous material, and a fresh thrombus 2 cm. from the

Fig. 15. Electrocardiogram of case K. A. was recorded continuously before, during and after an anginal attack by monitor ECU-Model 50 (Normal type).

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ostium was present for a distance of 2 cm. in the right coronary artery. The left coronary artery showed extreme narrowing, but was patent. Microscopic examination of the heart showed multiple old myocardial infarctions involving the anterior, lateral, and posterior walls of the heart. The anterior wall of the heart contained a moderate infiltration of polymorphonuclear white blood cells due to the ischemic changes of the heart muscle. Sections of the liver revealed congestion, caused by cardiogenic shock, as seen in Fig.25. These two fatal cases had a marked M-type electrophoretic pattern of SLDH isoenzymes.

Three additional patients in this series of 50 had increased LD1 activity (about 10%). One of these three had a fresh inferior wall myocardial infarction with an old anteroseptal myocardial infarction and A-V block. The other two had massive fresh anterior myocardial infarctions, complicated by congestive heart failure and ventricular tachycardia during treatment. These three patients with slightly increased LD1 activity had more severe complications than those with normal LD1 activity as seen in Fig.18. However, with careful treatment they recovered.

**DISCUSSION**

Recent developments in clinical research have established the presence of changing patterns of SLDH isoenzymes, as well as an increased level of SLDH activity, in the serum of patients suffering from various diseases. The commonest clinical application of the analysis of the pattern of SLDH isoenzymes has been in the diagnosis and prognosis of various diseases; it might be termed “chemical biopsy”. Other investigators have reported changes of the pattern of SLDH isoenzymes in many other physical disorders, in addition to myocardial infarction.

The author has analyzed the serial changes of the pattern of SLDH isoenzymes, as well as those of SLDH, SGOT, SGPT, and SCPK activities in patients with ischemic heart disease accompanied by chest pain, and correlated them with the clinical symptoms and serial changes in the electrocardiograms.

In angina of effort and the “normal type” of intermediate IHD, the SLDH isoenzyme pattern was N-type, and SLDH, SGOT, and SGPT activities remained within the normal range. In the “normal type” of intermediate IHD, however, SCPK activity was moderately increased in most cases. Although the author had no pathological material in cases of angina of effort and “normal type” of intermediate IHD, enzymological evidence suggested that in these cases the transient ischemic changes in the heart muscle were reversible and there was no necrotic change.
In the group showing the H-type pattern of SLDH isoenzymes, some patients had a transient increase of the LD5/LD4 ratio, and others a prolonged elevation. The former constituted the "abortive type" of intermediate IHD, and the latter had either fresh myocardial infarction or the "infarction type" of intermediate IHD with increased SLDH and various other serum enzyme activity. The "dissociation type" of intermediate IHD showed a prolonged H-type pattern of SLDH isoenzymes without increased SLDH activity. Patients with prolonged elevation of the LD5/LD4 ratio and total SLDH activity, (i.e. fresh myocardial infarction and "infarction type" of intermediate IHD), are considered to have myocardial necrosis following the attack, on the basis of clinical symptoms and electrocardiographic, laboratory, and autopsy findings.

The "dissociation type" of intermediate IHD with a prolonged H-type pattern of SLDH isoenzymes without increased SLDH activity resembled the cases reported by Wróblewski and Cohen. These cases were classified as subendocaridal infarction by Wróblewski and were thought by Cohen to be due to hyperpermeability of the cell membrane of the damaged heart muscle. The serial changes of the pattern of SLDH isoenzymes in this "dissociation type" were similar to those seen in fresh myocardial
Infarction, and therefore this "dissociation type" was considered reflect necrosis of the heart muscle, but the author had no pathological material to confirm this belief.

In the "abortive type" of intermediate IHD with a transient H-type pattern of SLDH isoenzymes after the anginal attack, it is presumed that a small amount of enzyme appears in the blood stream because of hyperpermiability of the cell membrane of the heart muscle or a small area of necrosis of the heart muscle. However, differentiation between hyperpermiability and a limited area of necrosis of heart muscle is not possible. As seen in case M.Y., the serial changes of the LD5/LD4 ratio of SLDH isoenzymes could be one of the most useful parameters in gauging the recovery from acute ischemic heart disease because it is increased again by exercise before it becomes normal, if myocardial ischemia is still present. Takayasu et al. reported the usefulness of SLDH isoenzymes in the rehabilitation of patients with acute ischemic heart disease. Further investigations should be carried out to clarify the serial changes of the pattern of SLDH isoenzymes in relation to the rehabilitation of much patients.

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Fig. 19. Serial changes of electrocardiograms taken after attack in case K. W. (Cardiogenic shock).

Fig. 20. Electrophoretic pattern of SLDH isoenzymes about 10 hours after attack in case K. W. (Cardiogenic shock).

Fig. 22. Serial changes of electrocardiograms in case T. K. (Cardiogenic shock).

Fig. 23. Electrophoretic pattern of SLDH isoenzymes about 4 hours after attack in case K. W. (Cardiogenic shock).

The appearance of an M-type pattern of SLDH isoenzymes in acute ischemic heart disease, reflects a grave prognosis. In the two fatal cases of fresh myocardial infarction, there was a marked increase of LD1 activity (about 30%), the so-called M-type pattern of SLDH isoenzymes, as seen in Fig. 18. Three other patients with acute ischemic heart disease had an
increased LD1 (about 10%), and complications such as A-V block, congestive heart failure, and ventricular tachycardia. These facts suggest that the M-type pattern of SLDH isoenzymes in acute ischemic heart disease could be one of the most useful indices of the severity of the disease. The results of the two autopsy cases suggest that the M-type pattern of SLDH isoenzymes may have its origin chiefly in the liver and skeletal muscle, due to acute circulatory failure. The

Fig. 24. Postmortem coronary angiography in case K. W.:
A) Right coronary angiography.
B) Left coronary angiography.

Fig. 25. Microscopic appearance of liver in case T. K. (Cardiogenic shock).

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damaged of the liver secondary to circulatory failure has been confirmed by the release of
SLDH isoenzymes from the liver. They may also be released from skeletal muscles as a result
of acute circulatory failure. Since the appearance
of LD1 activity is usually accompanied by
marked increase of SCPK activity which is very
sensitive to skeletal muscle damage, the release of
enzymes from skeletal muscle is quite possible.

It is interesting that, after the onset of the
attack, in the two who died, cases K.W. and T.K.,
the M-type pattern of SLDH isoenzymes
appeared 10 and 4 hours before the H-type
pattern, respectively. The reasons for this phe-

nomenon must be investigated, but one of the
reasons may be that a large amount of hepatic
tissue is injured by cardiogenic shock, in
comparison with a relatively limited lesion of the
myocardium.

**Summary**

The serum enzyme patterns of 50 patients
with acute ischemic heart disease accompanied
by chest pain were analyzed and correlated with
clinical and electrocardiographic findings. Two
autopsy cases are presented.

1) The patterns of SLDH isoenzymes were
obtained by the author's modification of "agar-
agarose gel electrophoresis”.

2) In patients with fresh myocardial infarction,
SLDH activity and the LD5/LD4 ratio began to increase 4 hours after the attack,
reached their peak in about 2 days, then
gradually decreased to normal, SLDH activity in
about 2 weeks, the LD5/LD4 ratio in 3 weeks,
on the average.

3) In patients with angina of effort, the
various serum enzymes remained within the
normal range.

4) Four types of intermediate IHD were
differentiated by their SLDH isoenzyme patterns:
infarction type, dissociation type (H-type
SLDH isoenzyme pattern without increased
SLDH activity), abortive type (transient H-type
pattern), and normal type.

The "infarction type” and “dissociation type”
were considered reflect necrosis of the heart
muscle. In the "normal type”, enzymological
evidence suggested that the ischemic changes in
the heart muscle were reversible. Only in the
"abortive type” it is unknown whether or
not a small area of necrosis is present. Thus,
ischemic heart disease accompanied by chest pain
can be more clearly subdivided into cases of
necrotic and non-necrotic heart muscle by
following the serial changes of the various serum
enzymes, especially the SLDH isoenzyme pattern.
in correlation with history, electrocardiograms,
and other laboratory findings.

5) Angina of effort and the “normal type”
of intermediate IHD were differentiated by
serum SCPK activity. In the “normal type” of
intermediate IHD, SCPK activity generally
increased after the anginal attack, but in angina of
effort, it remained within the normal range. Two
patients with increased SCPK activity of the
"normal type”, had the “variant form” of angina
pectoris as described by Prinzmetal.

6) The analysis of SLDH isoenzyme patterns
appears to be a very useful parameter in the
rehabilitation of patients with ischemic heart
disease accompanied by chest pain, but further
investigations are necessary.

7) The prognosis was poor in patients with
the M-type pattern of SLDH isoenzymes (i.e.
increased serum LD1) unless this was due to
complications with other diseases. All patients
with markedly increased serum LD1 (about 30%)
died. The M-type pattern of SLDH isoenzymes is
very useful in the diagnosis and treatment of
ischemic heart disease and probably originates
chiefly from damaged liver and skeletal muscles,
during acute circulatory failure.

8) In patients with severe IHD, the M-type
pattern of SLDH isoenzymes tends to appear
earlier than the H-type pattern. A probable
explanation of this phenomenon is that the
amount of hepatic tissue injured in cardiogenic
shock is very large in comparison with the
relatively limited lesion of the myocardium.

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**REFERENCES**

1. STRAUB, F. B.: Crystalline lactic dehydrogenase

2. NIELANDS, J. B.: Studies on lactic dehydrogenase

3. WIELAND, T. & PELEIDERER, G.: Nachweis der
Heterogenität von Milchsäure-dehydrogenasen ver-
schiedenen Ursprungs durch Trägerelektrophorese.

4. WIELAND, T. et al.: Über die Verschiedenheit der
Milchsäuredehydrogenase III. Vergleiche der Mil-

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