Chapter 5

CONCLUSION AND RECOMMENDATION

5.1 Experiments to Find Out the Optimum Conditions for Electrophoresis

5.1.1 Separation

A satisfactory separation of plasma proteins and LDH isoenzymes was obtained when the electrophoresis was carried out using 10 microlitres of plasma under the following conditions:

0.8 - 1.5% W/V Difco Special Agar-Noble in 0.05 M barbital buffer pH 8.6 at 24°± 1°C with a current of 7 mA./slide. The most satisfactory separation was obtained when the samples were applied at a distance 4 cm from the anodic site.

The least concentrated (0.8% w/v) and effective solution of the agar was used throughout. A more dilute solution of buffer could be used but a longer time was needed for the electrophoretic running. Fifteen minutes was required for the "prerun" process and the electrophoresis could be satisfactory completed in 1 hour.

5.1.2 Staining and destaining

A considerably good results was obtained when sodium lactate and glycine buffer, pH 8.7 were used instead of lithium lactate and glycine buffer pH 10 in the staining method for LDH isoenzymes described by Fritz et al (1970). Lipoproteins, simultaneously separated under the same conditions as LDH isoenzymes, were stained with the method of Dyerberg and Hjorne (1970). The isoenzymes and lipoproteins were destained

with 5% aq. acetic acid and 55% ethanol respectively.

5.1.3 Fixation

A mixture of methanol, water and glacial acetic acid in a ratio of 5:5:1 v/v was used for the fixation of LDH isoenzymes after the staining and destaining process. Proteins and lipoproteins had to be fixed immediately in a solution consisted of 10% acetic acid in methanol.

5.2 Quantitative Analysis of Plasma LDH Isoenzymes and Proteins in Normal Subjects.

Simultaneous analysis of plasma proteins and LDH isoenzymes showed that LDH-1, the most rapidly migrating isoenzymes lies between the albumin and the $alpha_1$-globulin. LDH-2 moves as far as the fastest part of $alpha_2$-globulin. LDH-3 lies between $beta_1$-and $beta_2$-globulin. LDH-4 is infront whereas the LDH-5 is behind the band of gamma-globulin.

Five bands of LDH isoenzymes were frequently found in the plasma of normal subjects but it was not uncommon to find patterns without LDH-4 and/or LDH-5.

5.3. Determinations of the Activity of LDH Isoenzymes

Determinations of plasma LDH isoenzymes activity in normal men (19 cases) and normal women (13 cases) of the age group between 25 and 45 years by spectrophotometric method showed that the TLDH activity in men plasma was slightly higher than in women plasma. There was no significant difference, however, in either the ULDH or the HLDH activity. The values were as follow:

	Men	Women
TLDH	121 ± 35	88 ± 12
ULDH	21 ± 11	12 ± 3
HLDH	12 ± 2	11 ± 2

With the aid of the densitometric method, the activity of the individual isoenzymes could be determined from the relationship of the area under peaks and the total activity obtained spectrophotometrically. The activities were found to be as follow:

	Men	Women
LDH-1	36 ± 9	26 ± 5
LDH-2	37 ± 11	27 ± 5
LDH-3	28 ± 10	20 ± 6
LDH-4	10 ± 3	7 ± 2
LDH-5	12 ± 6	7 ± 2

5.4 Studies on Changes of LDH Isoenzymes Activity in Patients with Myocardial Infarction

The studies were performed in the patients with the age group between 40 and 79 years. Activities of the isoenzymes in plasma samples taken at 1, 2, 3, 5, 10, and 15 days after the onset of the infarction were determined. An increase of the activity was observed within 24 hours and it reached to a maximum value between the third and the fifth days. The maximum activity was found to be 2 to 4 folds of that found in the plasma of normal subjects.

5.5 Determinations of Plasma Lipids

The levels found in normal subjects were as follows:

Concentration (mg /100 ml)	Men	Women
Cholesterol	271 ± 34	264 ± 40
Triglycerides	132 ± 42	136 ± 50

It was found that the electrophoretic conditions used for the separation of LDH isoenzymes did not give satisfactory results for lipoproteins.

5.6 Recommendation

The work in this thesis indicated that each method is valid under certain conditions. The measurement of either ULDH or HLDH activity is recommended as an aid in the enzymatic diagnosis of heart and liver disease particularly when results from other tests are equivocal.

The electrophoretic separation of the isoenzymes should be performed in complicate cases since much information would be obtained in comparisons with the measurement of HLDH, ULDH or TLDH activity. A combination of various methods is recommended, however, in cases which absolute certainty is required.

It would be interesting to study further

a. to find out optimum conditions for the electrophoretic separation of lipoproteins.

b. various substances present in plasma which interfere the determination of the isoenzymes activity.

c. the plasma isoenzymes activity in patients compare to that in a control group with similar age group.