

significant increase in the ovarian and spermathecal indices in *U. (celuca) lactea annulipes* after administration of 5-HT.

From the present investigation it is evident that both the eyestalk ablation and 5-HT have played an important role either acting directly or indirectly on the target organs and could have helped in the gonadal development.

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Immunochemical detection of serum LDH1: An indigenous method in the diagnosis of myocardial infarction

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Immunochemical studies on lactate dehydrogenase (LDH) were carried out to develop an indigenous immunochemical method for the diagnosis of acute myocardial infarction (AMI). Heart-specific LDH1 was purified and polyclonal antibody against LDH1 was raised in rabbits. Immunodiffusion and immunoblotting identifications show the presence of LDH1 in AMI test samples. Immunochemical detection of LDH1 in human serum may substitute kinetic method of total LDH assay and electrophoretic analysis of LDH1 isozyme in the diagnosis of AMI.

CORONARY heart disease is a major health problem in India and many other developing countries. It is more prevalent in urban than in rural areas¹. Thrombosis of a coronary artery typically occurs in the setting of coronary atherosclerosis and results in the clinical occurrence of acute myocardial infarction (AMI)². The diagnosis of AMI is usually predicated on WHO criteria of chest pain, electrocardiographic (ECG) changes, and increase in biochemical markers of myocardial injury³.

Cardiac enzymes are integral to the diagnosis and management of patients in whom acute coronary syndromes are suspected⁴. Because of differences in the

time that various markers have generally been used to identify its occurrence; CK-MB and isozymes of lactate dehydrogenase (LDH) have been widely used as early (prior to 24 to 36 h) and late markers (after 36 h), respectively^{5–8}. The increase of CK-MB persists for only 18–36 h and so cannot be relied upon to determine whether patients who present several days after a possible ischemic event have sustained AMI before presentation⁹. Hence, LDH1 and 3-hydroxybutyrate dehydrogenase (α -HBDH) remain of clinical significance for the non-invasive estimation of myocardial infarct size^{10–16}. LDH1 is slowly catabolised and a single measurement at 8 h represents measurement of total LDH1 release over 0–8 h period. Errors are smaller than more rapidly-cleared markers such as CK-MB, especially in patients with reperfusion therapy^{15–17}. It has also been indicated that LDH isozymes are as sensitive as cardiac troponin I (cTnI) values^{8,18}. The serial LDH1 levels are believed to be more specific for AMI than the LDH1/total LDH ratio^{6,8}. The problem of serial LDH1 assay may be minimized through immunochemical methods. Since no immunochemical test is available in India for the diagnosis of LDH1, the present study is intended to develop an immunochemical diagnostic methodology for the detection of serum LDH1 to improvise the assessment of myocardial infarction.

Control blood samples were obtained from 25 healthy and normal persons. Blood samples were also obtained from 30 patients admitted to Coronary Care Unit (12–20 h after onset of symptoms). These patients had ECG confirmation of AMI. Serum was separated from the blood samples with extra care to avoid haemolysis and

stored at 4°C for routine use. Non-denaturing polyacrylamide gel electrophoretic (non-SDS-PAGE) analysis was performed to study LDH isozyme patterns. LDH-specific staining on 4% non-SDS-PAGE comprised 0.1 M Tris-Cl buffer, pH 8.4; 1 mg \times ml⁻¹ NAD⁺; 0.5 mg \times ml⁻¹ NBT; 0.1 mg \times ml⁻¹ PMS and 0.05 M lithium lactate. The staining was done for 10–15 min in dark at 30 \pm 2°C. The gels were destained in 7.5% acetic acid.

Heart (left ventricular) tissue, free of disease, was obtained from two human cadavers within 8 h of death and stored at -80°C prior to use. They were healthy and did not have history of any disease prior to their death due to bullet injury. The heart isozyme of LDH (LDH1) was purified to its homogeneity by DEAE-cellulose chromatography¹⁹. Unless otherwise stated, all operations were performed at 0–4°C. A 20% homogenate (w/v) was prepared in 0.02 M Tris-Cl buffer, pH 7.4 containing 0.1 mM PMSF. The homogenate was centrifuged at 18,000 g for 30 min. The resulting supernatant was passed through glass-wool and clarified by recentrifugation at 18,000 g for 30 min. The supernatant fraction (crude extract) was chromatographed on DEAE-cellulose column (1.5 \times 35 cm). LDH1 was eluted through stepwise gradient in 0.02 M Tris-Cl buffer, pH 7.4 containing 0.3 M NaCl. The purity and homogeneity of the enzyme was checked using PAGE²⁰ and SDS-PAGE²¹.

Primary antisera against purified human LDH1 isozyme was raised in rabbits. The specificity and sensitivity of the antisera were examined by immunodiffusion and immunoblotting of the isozyme. Agarose (1%) plate for immunodiffusion was prepared in 0.1 M Tris-Cl buffer, pH 7.4 containing 0.9% NaCl. Unstained precipitin line(s) were seen after 8–12 h at room temperature. Crude extract and purified LDH1 were resolved on 4% non-SDS or 7.5% SDS-PAGE at 15 mA for 3 h. Gels were soaked in transfer buffer (0.025 M Tris base and 0.195 M glycine) for 30 min. Transfer was done at 4°C for 4 h at 200 mA or overnight at 14 V. No qualitative change was observed when repeated at 25°C. The transfer was monitored with Ponceau S staining. The membrane was processed for blocking for 2 h at room temperature in 0.1 M Tris-Cl, pH 7.5 containing 0.9% NaCl and 3% fat-free milk powder with intermittent shaking. Primary antibody (anti-rabbit human LDH1) and secondary antibody (anti-goat rabbit IgG-HRP conjugate) treatments were given for 2 h each in blocking buffer. The blots were washed in blocking buffer and 0.1 M Tris-Cl buffer, pH 7.5 containing 0.9% NaCl (10 washings, each at 10 min interval), respectively. Chromatogenic treatment (0.8 mg/ml DAB in 0.1 M Tris-Cl, pH 7.5 and 3 μ l of 3% H₂O₂) was given to develop the blot. After sufficient colour development, the membrane was thoroughly rinsed with distilled water, air dried and then photographed.

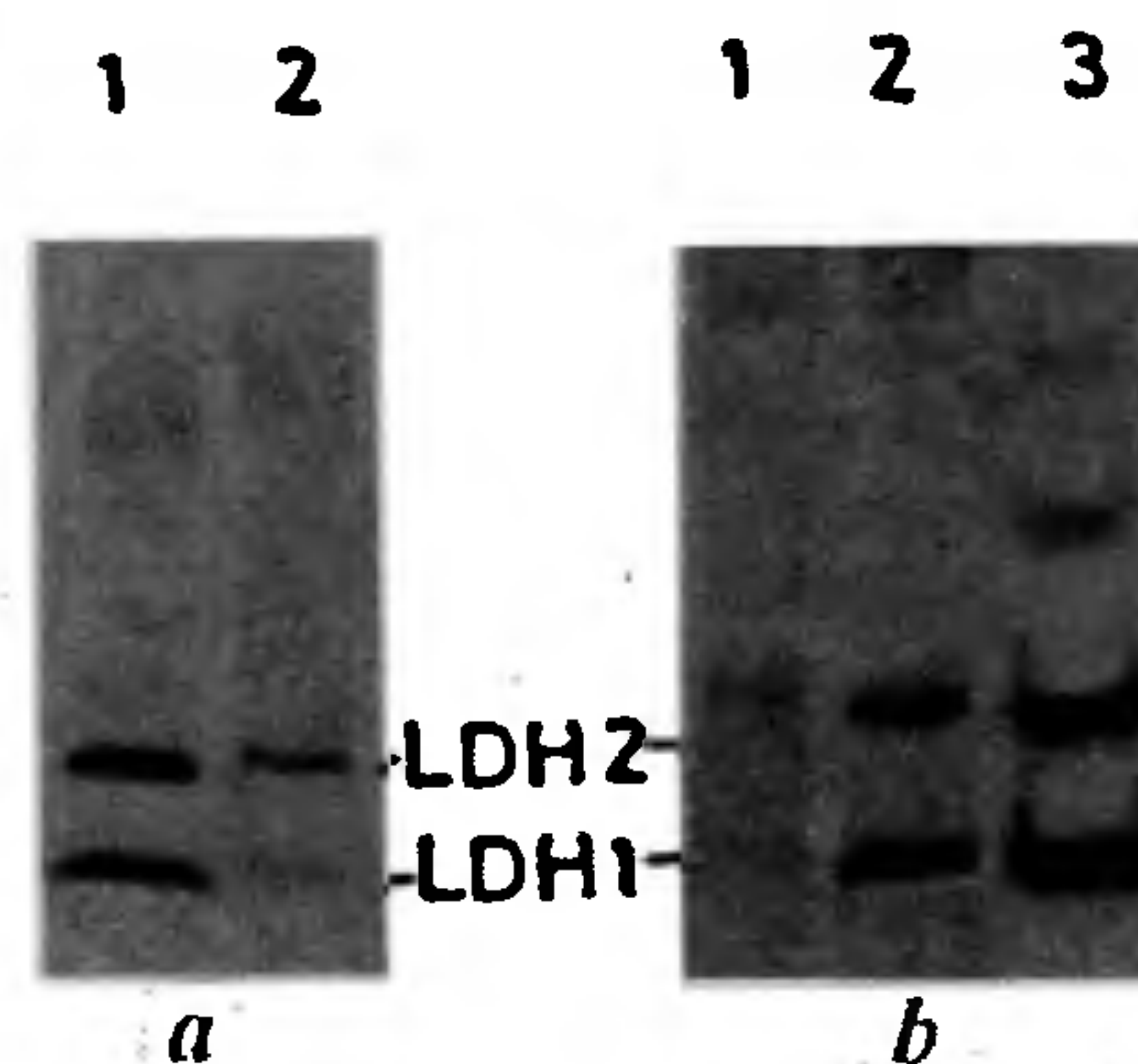


Figure 1. Detection of LDH in human serum based on specific staining after non-denaturing PAGE. Figure 1 *a* shows the presence of LDH1 and LDH2 in the serum of AMI patients. Lane 1, serum of AMI patient; Lane 2, serum of normal individual. Figure 1 *b* shows heart-specific LDH1 and LDH2 in the serum of AMI patient. Lane 1, serum of normal individual; Lane 2, serum of AMI patient; Lane 3, crude extract of human heart tissue.

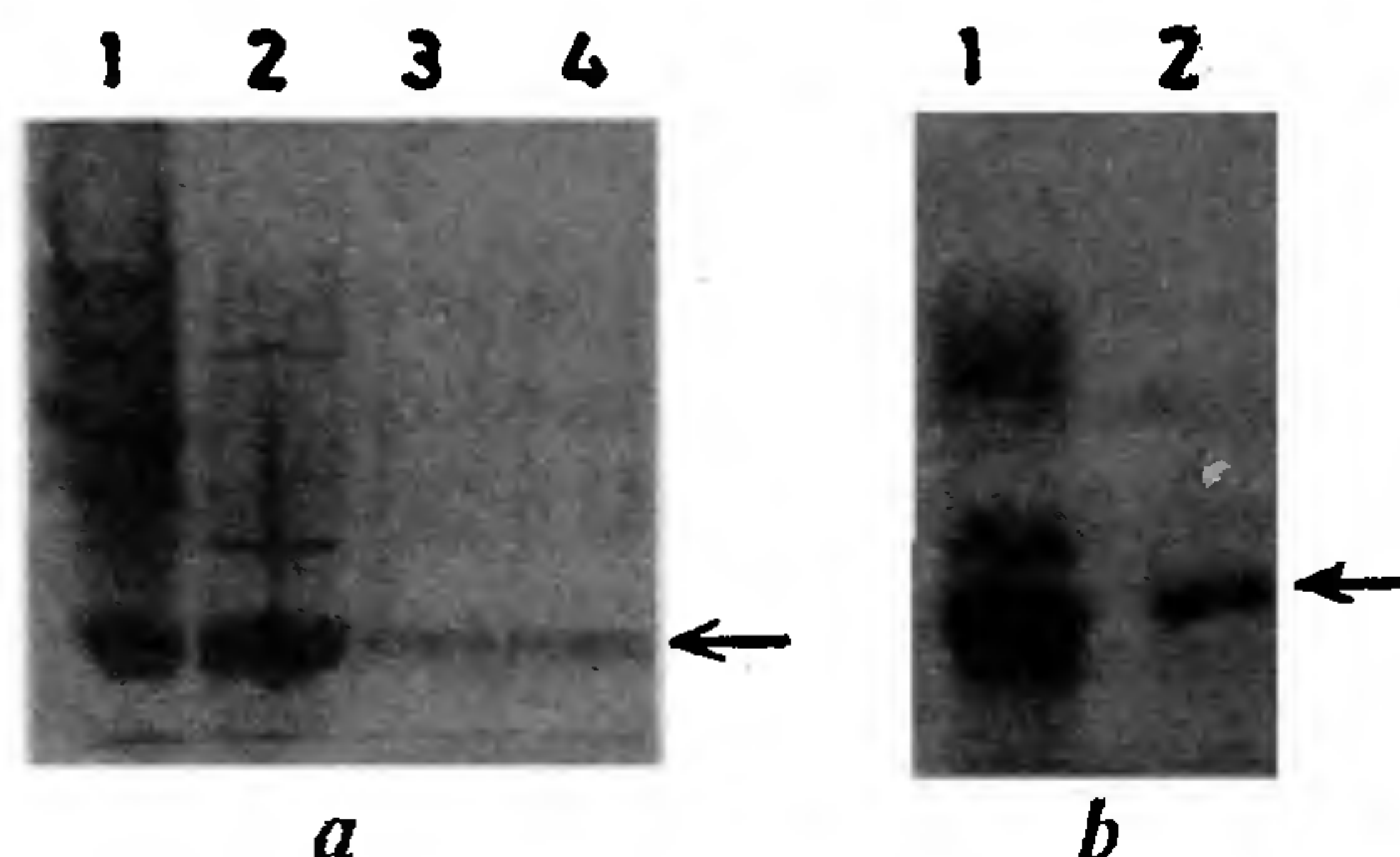


Figure 2. PAGE pattern of protein fractions obtained during purification of LDH1 from human heart tissue. (*a*) shows non-denaturing PAGE pattern on Coomassie brilliant blue R-250 staining. Arrows indicate the position of LDH1. Lane 1, Crude extract of human heart; Lanes 2–4, fractions from DEAE-cellulose column after stepwise gradient elution (0.3 M NaCl in 0.02M Tris-Cl, pH 7.4). (*b*) shows SDS-PAGE pattern of the protein fractions. Lane 1, crude extract of human heart tissue; Lane 2, purified LDH1 fraction.

Specific staining for LDH of human serum from normal individuals and AMI patients shows that LDH1 is predominant in the samples of AMI patients and LDH2 is also relatively high in such patients (Figure 1 *a*). Both isozymes correspond to heart-specific LDH isoenzymes (Figure 1 *b*). The mobilization of LDH1 and LDH2 during AMI suggests myocardial injury among these patients. The relative predominance of LDH1 to LDH2 is also an indication of AMI. Our findings are in agreement with earlier reports on reversal of LDH1/LDH2 ratio during AMI^{5,22}. Non-SDS and SDS-PAGE patterns of protein fractions taken at various steps of purification of LDH1 from human heart tissue show an apparent homogeneity of the purified sample (Figure 2 *a, b*). Immunodiffusion with antisera of LDH1 gave a



Figure 3. Immunodiffusion analysis with antisera to human LDH1 isozyme. A, antisera against LDH1; 1, crude extract of human heart tissue; 2, crude extract of skeletal muscle tissue; 3, purified LDH1 fraction; 4, pre-immune serum.



Figure 4. Immunoblot analysis with antisera to human LDH1 isozyme. Lane 1, crude extract of human heart tissue; Lane 2, purified LDH1 fraction.

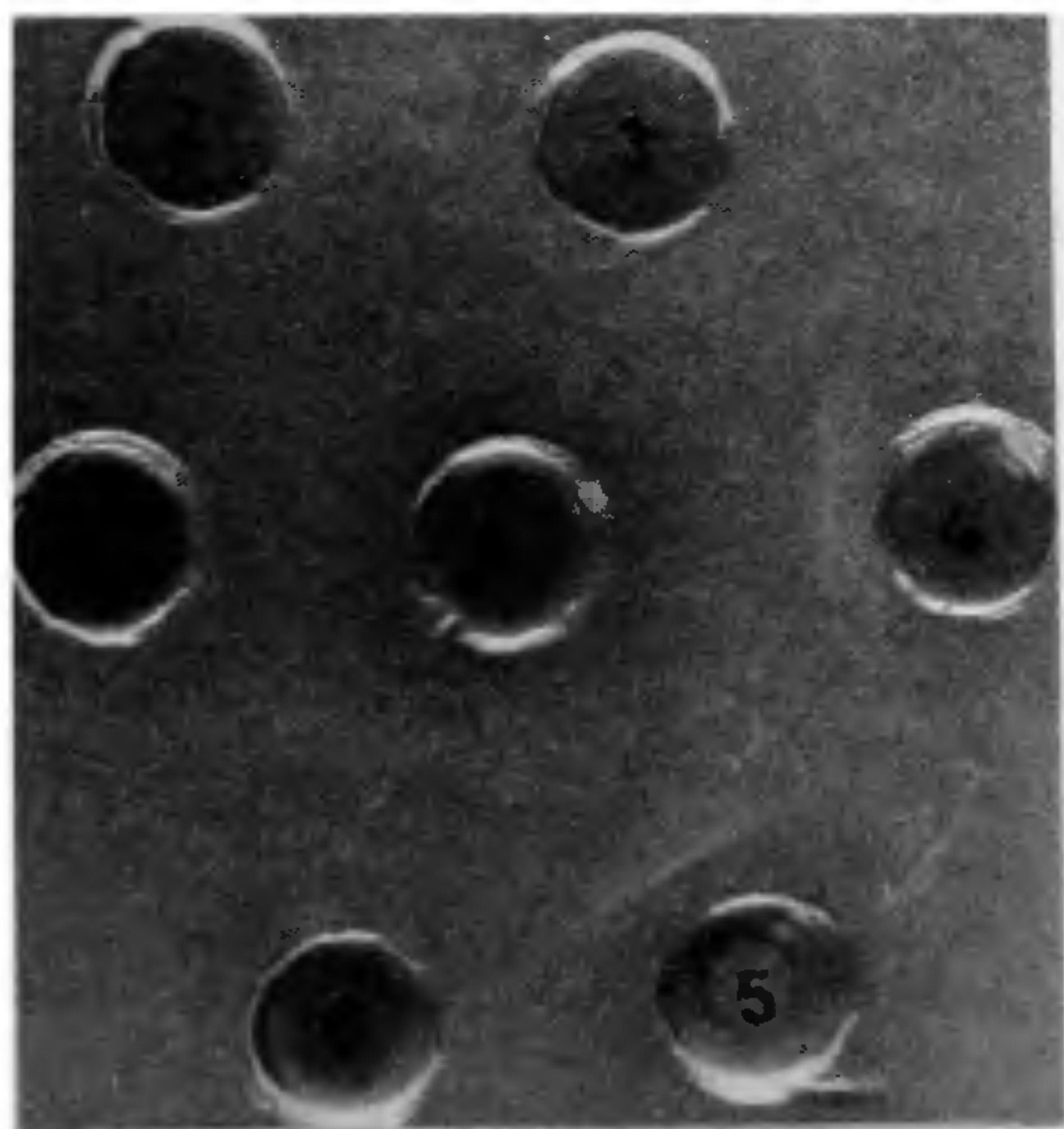


Figure 5. Immunodiffusion analysis with antisera to human LDH1 isozyme. A, antisera against LDH1; 1-3, serum of normal individuals; 4-6, serum of AMI patients.

strong precipitin line with purified LDH1 fraction and also shows crossreactivity with crude extracts of heart

and skeletal muscle tissues (Figure 3). Immunoblotting also recognizes LDH-specific polypeptide band on SDS-PAGE, though the antiserum was raised against native enzyme fraction. This type of immunochemical reaction may be due to some common epitopes between the native enzyme fractions and their respective polypeptides (Figure 4). Immunodiffusion with antisera of LDH1 gives precipitin lines with the serum of AMI patients and shows the presence of LDH1 in the serum of these patients (Figure 5). However, precipitin lines were not obtained in the serum of such patients with antisera against M₄-LDH. The use of antisera against M₄-LDH may avoid testing samples of persons having skeletal muscle dysfunctions. It will also discriminate the rise of serum LDH level which may be due to M₄-LDH of skeletal muscle origin. As compared to the duration of other tests, the time taken to complete the diagnosis is less than 10 min under present trial system on a strip. Its cost may also be many fold lesser than the cost of cTnI measurement⁴.

Strip-ELISA for the detection of LDH1 is being developed which may substitute for time and cost-effective diagnosis of myocardial infarction at the bedside, particularly in Indian conditions where patients are presented late in hospitals. It will also be useful in smaller hospitals having limited facilities for costly coronary angiography, echocardiography, or myocardial scintigraphy on 24 h basis.

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