Table 2 *Kinetic parameters of addition of acetone/ethylmethylketone and DNPH*

	Specific rate at 25.0°C ($k_2/M^{-1}\,Sec^{-1}$)	Energy of activation (E_a/kJ mol^{-1})	Frequency factor ($A/M^{-1}\,Sec^{-1}$)	Entropy of activation $\Delta S/JK^{-1}\,mol^{-1}$
Acetone	1.13	13.3	2.42×10^2	−207
Ethylmethylketone	2.83	46.8	4.52×10^8	− 87.5

Table 3 *Effect of acidity on the specific rate of the addition of acetone and DNPH*

Concentration of sulphuric acid/M	0.30	0.45	0.54	0.60	0.66	0.75
$k_2/M^{-1}\,Sec^{-1}$	1.13	0.51	0.36	0.27	0.22	0.15

Table 4 *Solvent effect on the specific rate of the addition of acetone and DNPH*

Percentage of 1,4 dioxane/%	0.0	5.0	7.5	10.0	15.0	20.0
$k_2/M^{-1}\,Sec^{-1}$	1.13	0.95	0.88	0.83	0.73	0.57

The various activation parameters evaluated are presented in table 2. The specific rate and the activation energy values in ethylmethylketone are higher than those in acetone. With increase in acidity the specific rate decreases (table 3). Similarly the specific rate decreases with increase in the percentage of dioxane (table 4).

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TWO DIMENSIONAL ELECTROPHORETIC DETECTION OF ABNORMAL SERUM PROTEIN IN PATIENTS WITH ENDOMYOCARDIAL FIBROSIS

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ENDOMYOCARDIAL fibrosis[1] (EMF) is one of the common causes of heart failure in the tropical world. It is an endemic disease in Kerala when compared to other parts of India and nearly 10% of all the cardiovascular cases in children admitted to hospitals in Kerala State are found to be EMF[2]. Geld *et al*[3] have reported the occurrence of antimyocardial antibodies in patients with EMF and this prompted us to analyse the serum proteins of these patients by high resolution two-dimensional (2-D) electrophoresis on polyacrylamide gel. Our study has yielded meaningful data regarding the abnormalities in the serum proteins of EMF patients and provided an approach for developing a tool for the diagnosis of the disease.

Twenty patients angiographically confirmed to be suffering from EMF were taken for this study. An equal number of age and sex matched controls were selected from the apparently healthy donors who came to the blood bank of our hospital. Sera separated from the blood samples collected from the patients and controls were used for electrophoresis immediately.

The electrophoretic procedure was described by few workers[4,5]. For the first dimension, 4.75%, 2% cross-linked, polyacrylamide gel cast in Corning tubes of length 9.5 cm and internal diameter 2 mm were used. An aliquot (10 μl) of the serum was loaded on each tube and the electrophoresis was carried out using trisglycine buffer, pH 8.3, at a constant current of 1.2 mA per tube until the tracking dye (bromophenol blue) reached the 1 cm mark above the anode end of the tube. For the electrophoresis in the second dimension a 2–30% linear gradient gel slab was cast in a mould of

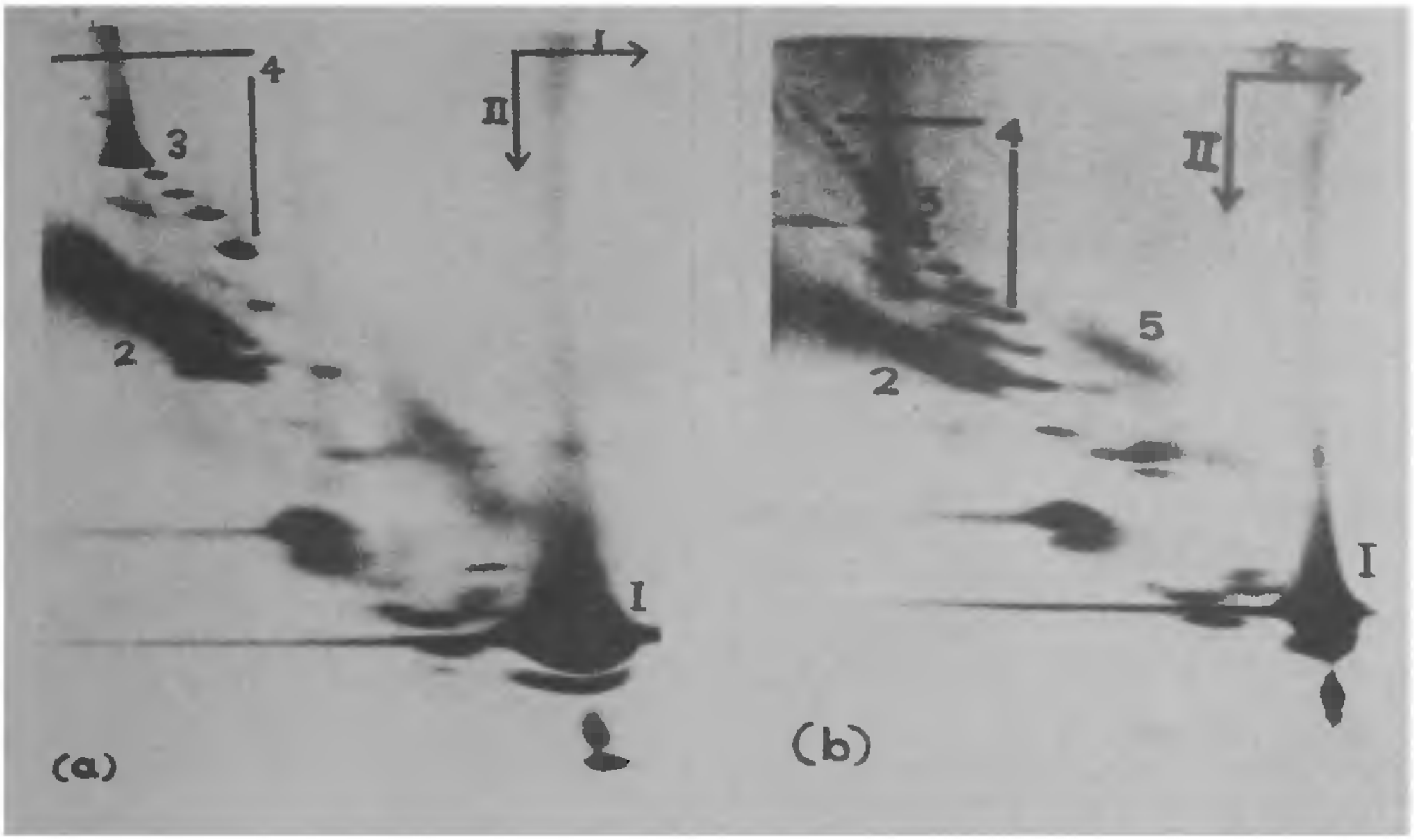

Figure 1a & b. 2-D electrophoretic pattern of serum proteins from control and EMF patient respectively. The horizontal and vertical arrows at the top right hand corner indicate directions of first and second dimensions of electrophoresis. Spots identified are: **1.** Albumin, **2.** IgG, **3.** α_2-Macroglobulin and **4.** Haptoglobin. **5.** Unidentified abnormal protein is numbered.

inner dimension 160 × 140 × 3 mm. The apparatus used for gradient gel electrophoresis and for the preparation of slab gradient gels were obtained from LKB-Produkter AB, Bromma, Sweden. The electrophoretic run was in trisglycine buffer pH 8.3, at 8–10°C, at a constant current of 20 mA per slab for a period of 22 hr. After the completion of the electrophoresis the slab gel was stained overnight in 0.2% Coomassie Brilliant Blue R 250 (Sigma Chemicals, USA) in 50% methanol 12% acetic acid[6]. The gel was destained with several changes of 10% ethanol 5% acetic acid. The destained gel was photographed and preserved in a sealed polythene bag containing a few drops of the destaining solution[7].

Typical 2-D electrophoretic patterns for the serum proteins from a healthy control and a patient with EMF are shown in figures 1a & b respectively. The spots corresponding to albumin, immunoglobulin IgG, α_2-macroglobulin and haptoglobins were identified by comparison with standard patterns. The intensity of the spots corresponding to haptoglobin and α_2-macroglobulins are found to be increased in all the EMF patients compared to controls. Although the albumin and IgG spots appear less intense in figure 1b the same trend is not consistently seen in all the cases we have compared. The most striking observation we wish to report is the occurrence of an abnormal protein represented by the prominent spot 5 in all the EMF patients' sera subjected to 2-D electrophoresis. We have also determined the amino acid composition of this protein by extracting the spots with 0.1% sodium dodecyl sulfate and hydrolysing the extract[8]. Amino acid analysis did not show the presence of any unusual amino acids in this protein. Further experiments are in progress to isolate and characterise this protein apparently important in understanding the biochemistry of the disease of EMF.

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ISOLATION OF A MICRO-ORGANISM FROM *PHILOSAMIA RICINI*

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RECENTLY the presence of urease has been shown in *Antheraea mylitta*[1] and *Philosamia ricini* (S. Kumar, personal communication). The synthesise of ascorbic acid has also been reported[2] for *P. ricini*. The ascorbic acid does not synthesise in insects which do not harbour symbionts. Similarly the presence of urease is indicative of the presence of a micro-organism in *P. ricini*, since urease is usually not present in asymbiotic insects. The present note reports the presence of a symbiont in *P. ricini*.

P. ricini was reared in the laboratory as described earlier[3]. The micro-organism was isolated from the fat body of *P. ricini* in sterile condition, by making a sharp incision in the pupae and making streaks of fat body in blood agar plates. Minute colonies grow in blood agar plates in 5–7 days. Different growth media were then used for growing the micro-organism thus isolated.

The micro-organism isolated was non-motile, gram-negative, rod-shaped and fairly uniform in size (0.3 to 0.5 μ). The growth in blood agar and nutrient broth was very slow; in 199 medium, the growth was better whereas in potato slant the growth was profuse. The micro-organism was negative for all the sugars and biochemicals used *viz* sorbitol, glucose, maltose, sucrose, salicin, lactose, trehalose, adonitol, mannitol, arabinose, mannose, inulin, dulcitol, dextrin, indole, citrate, gelatin and nitrate. The reaction with litmus milk was acidic.

The micro-organism isolated resemble *Wolbachia melophagi*, an extra-cellular symbiont of sheep ked, *Melophagus ovinus*.

The presence of symbiont in silk worms has not been reported so far. Its isolation from *P. ricini* explains many anomalous results reported for *P. ricini*. Thus the reported results that cholesterol and ascorbic acid synthesis take place in *P. ricini*[2,4] can be explained. The presence of urease in *P. ricini* can also be similarly explained.

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GREEN MOSAIC: A VIRUS DISEASE OF *HYOSCYAMUS MUTICUS* L. IN INDIA

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DURING 1981–82 *Hyoscyamus muticus* L. (Egyptian henbane), one of the important medicinal crops was severely affected by mosaic disease in the experimental farm of CIMAP at Lucknow. The diseased plants showed severe stunting and bushy appearance. The leaves were reduced in size (figure 1-A) and sometimes narrowing of leaves into shoe lace was also recorded. In general, mosaic mottling symptoms were more pronounced in young leaves. Often the flowers became discoloured and seed setting was poor. A systematic study of this new disease was made in terms of transmission, host range, virus purification and some properties of the virus *in vitro*.

Mechanical transmission studies conducted in the glass-house (15–35°) revealed that the disease was readily transmissible through sap. The sap was extracted from the mosaic infected leaves in 0.1 M