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DISTINCT DEHYDROGENASE ISOENZYMES IN STRAINS AND SPECIES OF SIMIAN MALARIAL PARASITES AS EVIDENT BY GRADIENT POLYACRYLAMIDE GEL ELECTROPHORESIS

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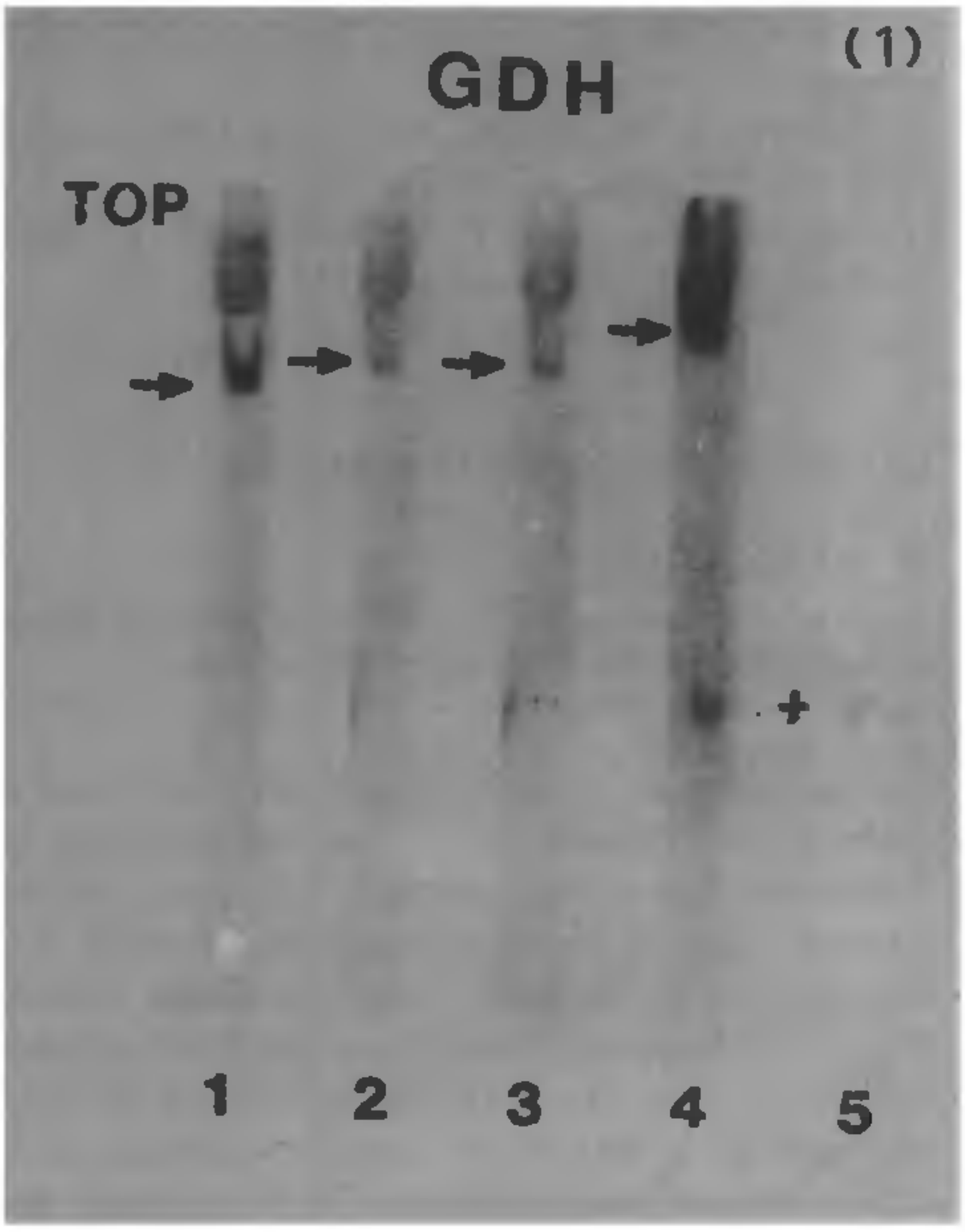
ENZYME electrophoresis is a valuable tool for identifying genetic variations among different species and strains of parasites[1–3]. Among malarial parasites, the technique was first applied to rodent species of *Plasmodium*[4,5] and subsequently to human and primate malarial parasites[1,6,7]. Earlier studies on isoenzymes were mainly confined to employing starch gel or agarose gel electrophoresis. Polyacrylamide gradient gel electrophoresis, which yields much greater resolution of proteins in comparison to other supports like starch or agarose, has recently been used successfully for isoenzymic studies of malaria[7], leishmaniasis[8] and amoebiasis[9]. The present report describes the isoenzymic patterns of glutamate dehydrogenase (E.C.1.4.1.4, GDH) and malate dehydrogenase (E.C.1.1.1.37, MDH) of simian malarial parasites, separated on polyacrylamide gradient gel electrophoresis.

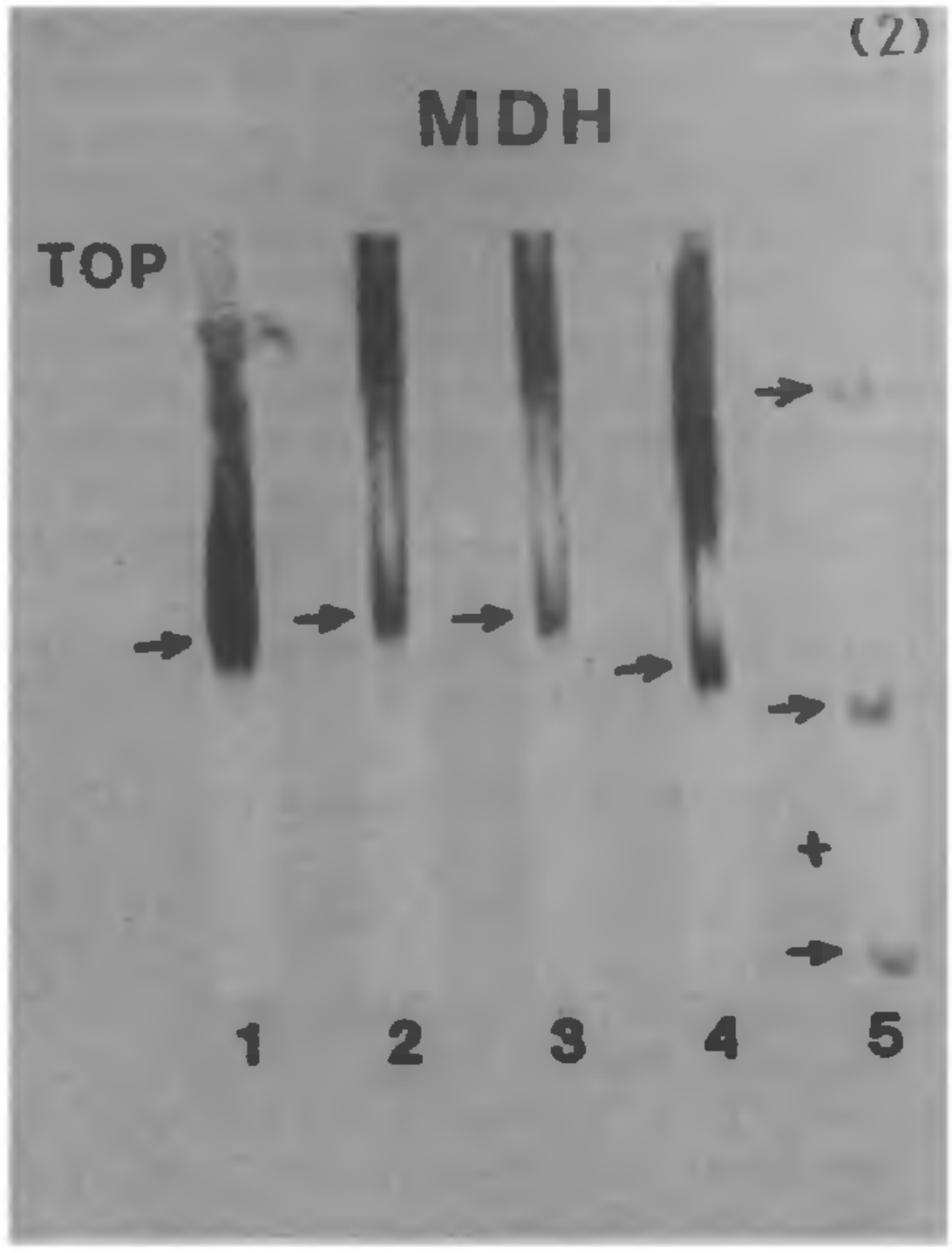
Two species of simian malarial parasites viz. *Plasmodium knowlesi* and *P. cynomolgi bastianelli* were used. Three strains of *P. knowlesi*, one from Philippines (P-strain) and the other two from Malaysia (H-strain and W_1 strain), which are known to induce fatal infections in rhesus monkeys were used. *P. knowlesi* P and H strains were kindly provided by Dr L. H. Miller, Malaria Section, Laboratory of Parasitic Diseases, NIH, Bethesda, MD, USA. *P. knowlesi* W_1 strain was obtained from Prof. P. C. C. Garnham, Imperial College, London, UK. The other species of simian malaria, *P. cynomolgi* B producing less severe non-fatal infection in rhesus monkeys was obtained from Dr William Collins, Centre for Disease Control, Atlanta, Georgia, USA. Both these species also infect man.

Rhesus monkeys (*Macaca mulatta*) of either sex weighing about 3–6 kg were infected by intravenous infusion of 1×10^6 schizonts of *P. knowlesi*/*P. cynomolgi* and the percentage of parasitaemia was determined by examining Giemsa stained thin blood films. Blood from infected monkeys was collected in acid citrate dextrose when most of the parasites were at schizont stage. The schizonts were purified on Percoll gradient and extracted as described earlier[7]. The enzyme activities of GDH[10] and MDH[11] were determined. The protein contents of the parasite and normal erythrocyte ghost extracts were estimated by the method of Lowry *et al*[12] as modified by Deans *et al*[13].

Polyacrylamide gel electrophoresis of parasite and normal erythrocyte extracts was done under non-dissociating conditions following Davis[14] with certain modifications. The separating and the stacking gels were used as described earlier[15]. 5.0–7.5% separating and 3% stacking gels (without sodium dodecyl sulphate) were made in 0.375 M Tris-HCl buffer pH 8.8. Electrophoresis was carried out at 20 mA for 12–14 h in the case of GDH and 18–20 h in the case of MDH. The temperature was maintained at 4–8°C with a cold water bath circulator. Specific staining of MDH and GDH was done as described by Carter[4,5].

In the present study, the technique of polyacrylamide gradient gel electrophoresis has been used to study the isoenzymic patterns of GDH and MDH of different strains and species of simian malarial parasites. Electrophoresis was done for varying lengths of time to get better separation of the isoenzymic bands. A running time of 12–14 h was sufficient to separate the GDH isoenzymes as further increase in the running time did not affect the separation of the isoenzymic bands though it did result in some loss of GDH activity. On the other hand, for getting better separation of MDH isoenzymes, the gel had to be run for 18–20 h. Zymograms of GDH and MDH are shown in figures 1 and 2 while figure 3 shows the diagrammatic representation of the same. Only one major isoenzymic band of GDH in *P. cynomolgi* and different strains of *P. knowlesi* was observed, but they differ in their





Figures 1 and 2. 1. Isoenzymic pattern of glutamate dehydrogenase. 1. *P. knowlesi* P strain; 2. *P. knowlesi* H strain; 3. *P. knowlesi* W_1 strain; 4. *P. cynomolgi* B; 5. Normal monkey erythrocytes; (+) Haemoglobin; (→) Isoenzymic band, and **2.** Isoenzymic pattern of malate dehydrogenase. Details are as in figure 1.

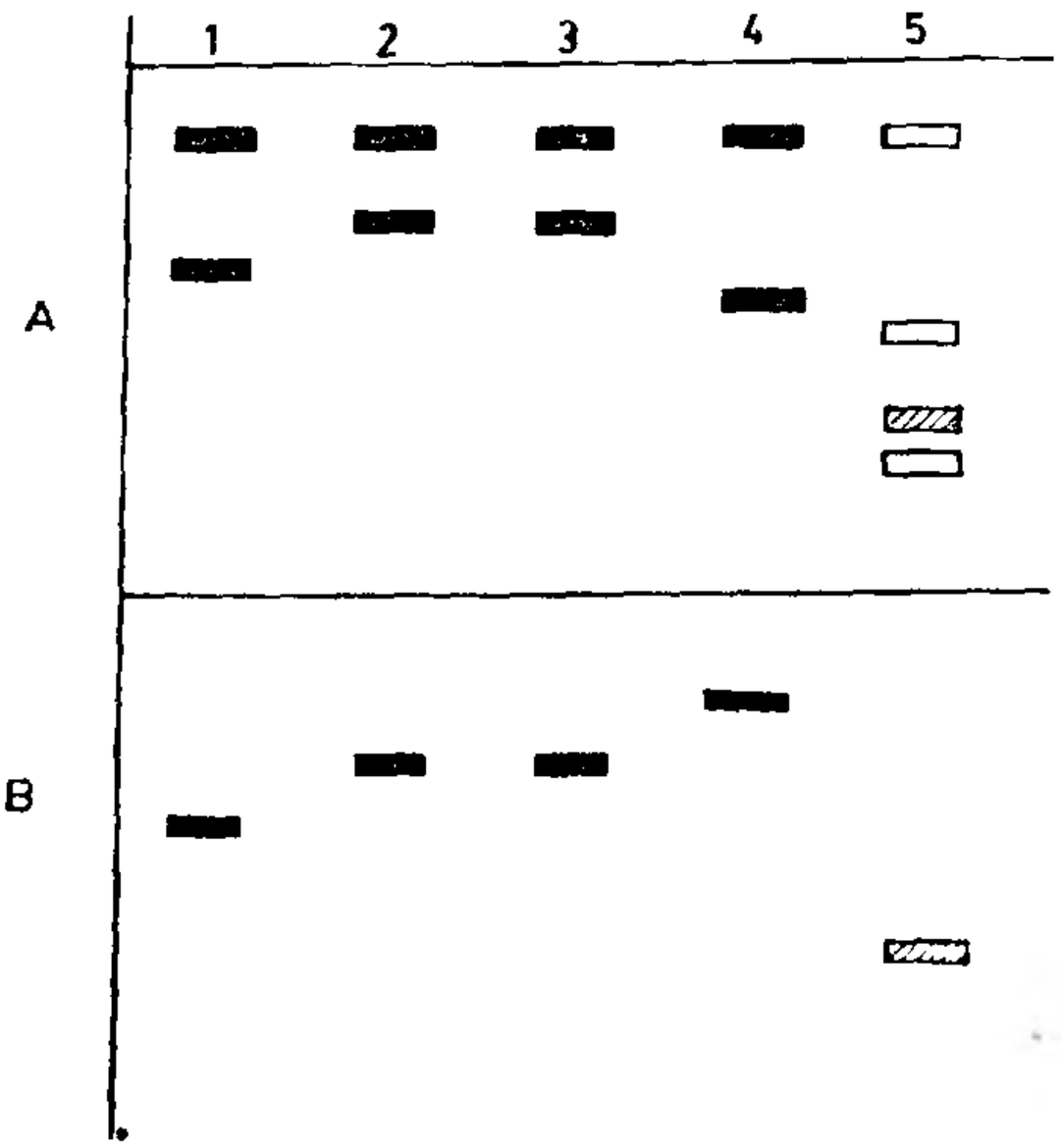


Figure 3. Diagrammatic representation of isoenzymic pattern of malate dehydrogenase (A) and glutamate dehydrogenase (B). Details are as in figure 1. [■ Parasite enzyme; □ RBC-enzyme; ▨ Haemoglobin.]

electrophoretic mobilities. *P. cynomolgi* and *P. knowlesi* could also be differentiated from one another on the basis of MDH isoenzymes. There appeared to be two isoenzymes of MDH in these parasites, but only one (fast moving) showed variation in electrophoretic mobility between the strains and species of these parasites. The other isoenzymic band (slow moving), though found different in *P. cynomolgi*, was not very distinct in all the strains of *P. knowlesi*. Moreover, it was not distinguishable from the top isoenzymic band (slow moving) of the host (normal RBC).

On comparison, isoenzymes of GDH and MDH of different strains of *P. knowlesi*, P-strain (Philippine strain) appeared to differ from other strains, H and W_1 (Malaysian strains) in their electrophoretic mobilities. Normal erythrocyte showed three isoenzymic forms of MDH while no isoenzymic band for GDH was observed.

As stated earlier, the three strains of *P. knowlesi* used originated from two different geographical regions i.e. Philippines and Malaysia. These strains are known to be antigenically and morphologically similar[16]. However, in the present study we are able

to differentiate the Philippine strain from the Malaysian ones on the basis of the isoenzymic patterns of GDH and MDH. These strains have also been differentiated earlier on the basis of LDH isoenzymes[3]. Differences in the circumsporozoite protein genes of the two strains (P and H) were also observed[17]. These findings suggest that the parasites from the two different geographical regions are genetically different and possess distinct isoenzymic pattern. Carter also differentiated subspecies of murine malaria by isoenzymic studies on MDH and GDH[4,5].

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RADIOACTIVE DINOSAUR BONES

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DINOSAUR bones[1–4], teeth[5] and eggs[6–9] are found in Cretaceous-? Palaeocene[10,11] Lameta sediments of Kheda and Panchmahal districts of Gujarat. During a recent study of dinosaur bones from Rahioli area (figure 1) in the Balasinor taluk of Kheda district, Gujarat, the present author found them to be highly radioactive. So far there is no record of high radioactivity in either the Lameta sediments or in other dinosaur fossils of India. The U content in the dinosaur bones from Lameta sediments from Umrer (Nagpur) and Jabalpur ranges between 66 and 246 ppm[12]. However, the mammalian fossils in the Upper Siwalik Formation of Pinjor Himalaya are known for noticeable concentration of uranium[13–15] (0.05–0.34% U_3O_8 with no thorium). Outside India, the dinosaur bones from Morrison Formation (Jurassic) of Camp Davies Region, Western Wyoming (USA) are also known for their radioactivity[16] (0.04–0.12% U_3O_8). The presence of radioactivity in the dinosaur bones of Lameta sediments is of great significance as it may serve as a guide for locating deposits expected to contain radioactive mineral in commercial quantity. So far, the only other sedimentaries which are time contemporaneous to Lametas and also known for uranium concentration are the Mahadeo (Mahadek) Formation of Khasi and Jaintia hills of Eastern India[17,18].

The uraniferous dinosaur bones were collected from the eastern slope of a north-south trending hillock, about 1 km west of Rahioli village. In this area, the sedimentary sequence comprising greenish conglomerates/grits, greenish grey, medium to fine-grained calcareous sandstone and fine-grained, mottled to purplish arenaceous limestone belong to Lameta Group (figure 1). The sedimentaries overlie the Precambrian Godhra granite and pegmatite. The Deccan Trap volcanics which overlie the Lameta sediments elsewhere, have been eroded in