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# Haptoglobin Phenotypes in Leprosy[1]

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Leprosy continues to fascinate the biologist because of the unusual host-parasite interactions. The parasite, indolent, slow-growing, and without aggressive weapons, continues to defy attempts at *in vitro* cultivation. Interestingly enough, it is the host-response that determines whether clinical disease will be tuberculoid or lepromatous. Attempts have therefore been made to probe the genetic markers of the host in the hope of defining not only susceptibility to leprosy but also correlations with the clinical varieties. These include studies of pseudocholinesterase ([15]), HLA-types ([5]), blood group antigens ([9, 10]), haptoglobins, transferrins, and glucose-6-phosphate-dehydrogenase phenotypes ([8]).

Haptoglobins are plasma proteins with the property of combining with hemoglobin. They show a genetic polymorphism recognizable by electrophoresis because of the genes $Hp^1$ and $Hp^2$ as the four phenotypes 0-0 (absence), 1-1, 2-1, and 2-2 ([14]). Data for normal communities in India reveal only small variations in gene frequencies ([1, 2, 16]). To our knowledge, the world literature describes only two reports of haptoglobin phenotypes in leprosy ([8, 10]), and there is none for leprosy patients on the Indian subcontinent. We here describe such a study.

## MATERIALS AND METHODS

**Cases.** Eighty cases of leprosy attending the Outpatient Department of the Sir J. J. Group of Hospitals, Bombay, were studied. A detailed clinical history, including duration of disease, occurrence of reaction, nature and duration of treatment, and a careful clinical examination was carried out in each case. The bacteriological and morphological indexes were determined ([6, 11]). A skin biopsy was taken and studied histologically. Each case was then classified according to the scheme of Ridley and Jopling ([12]). A sample of serum was obtained and used to determine the haptoglobin phenotypes.

**Controls.** Sera obtained from 100 normal, nonleprosy volunteers were studied for haptoglobin phenotypes.

**Determination of haptoglobin phenotype.** This was done in polyacrylamide disc gel electrophoresis, using a miniaturized system ([4, 13]). Freshly prepared human red cell hemolysate (0.01 ml of a 10 g/dl solution) was added to 0.25 ml of the serum sample and 0.01 ml used to inoculate the column ([7]). Gel columns were 7 cm high, 2 mm in diameter, and made from 7 percent monomer in a 0.1 M tris-glycine buffer, pH 8.3, without sodium dodecyl sulfate. Electrophoresis was carried out with a current of 1 mA per column for about 30 min, the run being monitored with a bromophenol blue marker. The hemoglobin-haptoglobin complexes were stained by the benzidine reaction ([7]) (The Figure).

**Special studies of cases with haptoglobin phenotype 0-0.** Wherever possible, cases showing haptoglobin phenotype 0-0 were studied to rule out hemolysis as a cause of anhaptoglobinemia. These tests were: examination of a peripheral blood smear, reticulocyte count, red cell osmotic fragility, test for sickle cells, electrophoretic study of hemoglobin for abnormal hemoglobins, and test for red cell glucose-6-phosphate dehydrogenase deficiency.

## RESULTS

The Table summarizes the results. Statistical analysis showed that the occurrence of the phenotypes in terms of age or variety of leprosy except for phenotype 0-0 was not significantly different from the controls. Haptoglobin phenotype 0-0 was more frequent in leprosy ($p < 0.02$). The five cases showing phenotype 0-0 were all males on prolonged dapsone (DDS) therapy, and their ages were 20, 24, 30, 40, and 60 years

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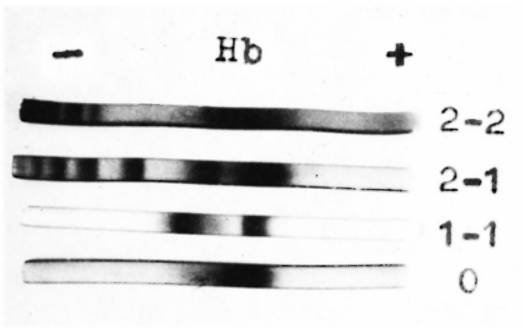
respectively. Three had lepromatous leprosy in reaction, and two had tuberculoid disease. One of the cases of tuberculoid leprosy had had jaundice (presumably infectious hepatitis) 3 months earlier. Two of the five cases of 0-0 phenotype could be followed up a month later, and surprisingly enough, one showed the appearance of weak but unequivocal bands of the 2-1 phenotype, indicating that this patient had had a secondary anhaptoglobinemia. Hematological studies in these two cases failed to reveal any evidence of a hemolytic process of other hematological abnormality.




THE FIGURE. Polyacrylamide disc gel electrophoretic separations of human serum stained by the benzidine reaction to demonstrate haptoglobin phenotypes. Free hemoglobin is the fastest-moving component. The 2-2 pattern shows several slow-moving bands. The 1-1 pattern has only a single fast-moving band a little slower than the free hemoglobin. The 0-0 type shows the absence of any haptoglobin bands, and only the free hemoglobin component is seen.

## DISCUSSION

Genetic susceptibility to leprosy and the clinical variety of the disease has been the subject of much study. So far, however, no satisfying correlations have been established. The problem was lucidly discussed by Blumberg and Melartin [3], who pointed out that the susceptibility (S) factors might be multiple, of the nature of either dominant or recessive traits, operating differently at various ages, and expressing themselves through phagocytic cells, the reticuloendothelial system, and the immunological apparatus. The authors suggested that $Au_1$, a recessive character controlling susceptibility to symptomless carriage of the hepatitis-B virus might be one such factor. Blood group systems have been the subject of much study, but no significant correlations have been established [9, 10]. Thomas and Job [15] described atypical serum pseudocholinesterases in leprosy patients below 40 years of age. de Vries, *et al.* [5] described an association between some of the cell membrane antigens of the HLA-D system and susceptibility to leprosy.

With respect to haptoglobin phenotypes, Povey and Horton [10] found no significant correlations. In contrast, Lechat, *et al.* [8], studying several genetic polymorphisms in leprosy in the Philippines, found that the haptoglobin phenotype 1-1 was significantly more frequent in both lepromatous and tuberculoid leprosy patients. The phenotype 2-1 was more frequent in leprosy patients over 40 years old. This was interpreted to indicate that either the phenotype led to a more persistent type of disease or to preferential survival if it was not a sampling artifact.

Our study compares with that of Povey and Horton [10]. There were no significant

THE TABLE. *Haptoglobin phenotypes in leprosy.*

| Haptoglobin phenotype | Normal controls | | | Leprosy—all forms | | | Leprosy classification[a] | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | <40 yr | >40 yr | Total | <40 yr | >40 yr | LL | BL | BT | TT |
| 2-2 | 71 | 55 (66)[b] | 16 (94) | 52 (65) | 30 (65) | 22 (65) | 23 (60) | 9 (82) | 5 (83) | 14 (59) |
| 2-1 | 27 | 26 (31) | 1 (6) | 22 (27.5) | 12 (26) | 10 (29) | 12 (32) | 2 (18) | 1 (17) | 7 (29) |
| 1-1 | 2 | 2 (3) | 0 | 1 (1.25) | 0 | 1 (3) | 0 | 0 | 0 | 1 (4) |
| 0-0 | 0 | 0 | 0 | 5 (6.25)[c] | 4 (9) | 1 (3) | 3 (8) | 0 | 0 | 2 (8) |
| Total | 100 | 83 | 17 | 80 | 46 | 34 | 38 | 11 | 6 | 24 |

[a] There was a single case of indeterminate leprosy with haptoglobin phenotype 2-2 and over 40 years of age. The patients were classified according to the method of Ridley and Jopling [12].

[b] Numbers in parentheses indicate percentage values with respect to that column.

[c] $p < 0.02$ compared to normal controls.

differences in haptoglobin phenotypes in leprosy patients and controls except for the increase in the 0-0 phenotype in the leprosy patients. The 0-0 phenotype expresses as a total absence of haptoglobin in the serum (The Figure). In veiw of the change of phenotype from 0-0 to 2-1 in one of the two cases in which a follow-up was possible, it is possible that the predominance of the 0-0 phenotype in leprosy is actually due to secondary anhaptoglobinemia. One plausible reason for secondary anhaptoglobinemia, a hemolytic process, could be ruled out. However, it is possible that other factors such as drug therapy and reaction might cause a secondary anhaptoglobinemia. Our knowledge of haptoglobins and their role in the economy of the "*milieu interior*" is so limited that speculations may be unwarranted. In any event, such individuals with anhaptoglobinemia (either primary, phenotype 0-0, or secondary) would be prone to develop hemoglobinuria if they experience hemolysis because of the absence of this hemoglobin-combining protein in the blood stream.


## SUMMARY

Serum haptoglobin phenotypes were studied in 80 patients with leprosy classified according to the criteria of Ridley and Jopling. The distribution of phenotypes was: 2-2, 65%; 2-1, 27.5%; 1-1, 1.25%; and 0-0, 6.25%. This distribution was not significantly different from the controls except for the phenotype 0-0 ($p < 0.02$). Thus, although this genetic marker did not correlate with the occurrence of the variety of disease, it is possible that leprosy caused inhibition of haptoglobin synthesis and therefore an apparent increased frequency of the 0-0 phenotype. Evidence for such a secondary anhaptoglobinemia was available in one case.

## RESUMEN

Se estudiaron los fenotipos de la haptoglobina sérica en 80 pacientes con lepra clasificados de acuerdo a los criterios de Ridley y Jopling. La distribución de los fenotipos fue: 2-2, 65%; 2-1, 27.5%; 1-1, 1.25% y 0-0, 6.25%. Esta distribución no fue significativamente diferente de la de los controles, excepto por el fenotipo 0-0 ($p < 0.02$). Aunque este marcador genético no correlacionó con una forma particular de la enfermedad, es posible que la lepra haya causado inhibición de la síntesis de haptoglobina y por lo tanto, una frecuencia aparentemente aumentada del fenotipo 0-0. En un caso hubieron evidencias de dicha anhaptoglobinemia secundaria.

## RÉSUMÉ

Chez 80 malades atteints de lèpre et classés suivant les critères de Ridley et Jopling, on a étudié les phénotypes des haptoglobines du serum. La distribution des phénotypes était la suivante: 2-2, 65%; 2-1, 27.5%; 1-1, 1.25%; et 0-0, 6.25%. Ces distributions n'étaient pas significativement différentes de celles observées chez les témoins, sauf pour ce qui est du phénotype 0-0 ($p < 0.02$). Dès lors, quoique ce marqueur génétique ne présente pas de corrélation avec le type de la maladie dont souffre le malade, il est possible que la lèpre produise une inhibition de la synthèse des haptoglobines, qui se traduirait par une augmentation apparente de la fréquence du phénotype 0-0. Dans un cas, on a trouvé des signes qui suggéreraient une anhaptoglobinémie secondaire.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