PHENOTYPIC EXPRESSION OF THE Hp^2/Hp^1 GENOTYPE

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In man, three major haptoglobin phenotypes, designated 1-1, 2-1, and 2-2 have been described (Smithies and Walker, 1956). All are built of α and β polypeptide chains. These major classes differ in their α chains (Smithies, Connell and Dixon, 1962, 1962a). α^1 chains, which can be further classified into α^{1F} and α^{1S} are coded for by the Hp^1 genes, and the doubly long α^2 chains by the fusion gene Hp^2. Homozygotes expressing 1-1 and 2-2 phenotypes have only α^1 and α^2 chains respectively. Heterozygotes contain both α^1 and α^2 chains in approximately equal numbers. The β chains of each major type are identical and are coded by a gene with a locus distinct from that coding α chains. The major types can be distinguished electrophoretically, for the 2-1 and 2-2 haptoglobins form characteristic polymers and the 1-1 type occurs only as a monomer (Smithies 1955).

According to Allison (1959), each term in the 2-1 series contains only one unit of Hp1 per molecule. Starting at the second term Hp2 units are incorporated, one further unit being added to the molecule with each successive term. It is thought that certain quantitative differences in the expression of the Hp^1 and Hp^2 genes are reflected in ratio

variations between terms of the 2-1 series. It has for instance been shown that sera of 2-1M phenotype, in which relative underproduction of the α^2 polypeptide chain occurs, have a preponderance of the lighter α^1 rich term (Parker and Bearn, 1963).

Observations made in our laboratory using polyacrylamide gel electrophoresis in a continuous molecular sieve gradient have shown the presence of further bands corresponding to polymers of the 2-2 series in sera taken from normal people of Hp^2/Hp^1 genotype.

Continuous concave polyacrylamide gel gradients (see Fig. 1) cast in disposable glass cells of internal diameter 83 x 73 x 3 mm were prepared in batches according to methods previously described (Margolis and Kenrick 1967, 1968; Margolis, 1969). With the aid of spacers, 5µl. samples of undiluted sera mixed with haemoglobin sufficient to saturate haptoglobin, were applied. The gels within their cells were then placed in the electrophoresis chamber filled with Tris/EDTA/Boric acid buffer pH8.3 (Kitchin, 1965), and run for 24 hours at 75 volts and 6-8°C with current falling from 10 mA/cm^2 to 4 mA/cm^2. They were then removed from their cells, stained with 0.3% Amido Black in acetic acid: methanol: water (1:3:6) for at least 8 hours, and destained for a further 48 hours in 5% acetic acid. Other slabs were sliced parallel to their faces and stained with benzidine (Smith, 1968).

In all sera belonging to the Hp2-1 type, both Amido Black and benzidine stains reveal the presence of small amounts of the first three terms of the Hp2-2 series (see Fig. 1). Additional bands have not been demonstrated in sera of either

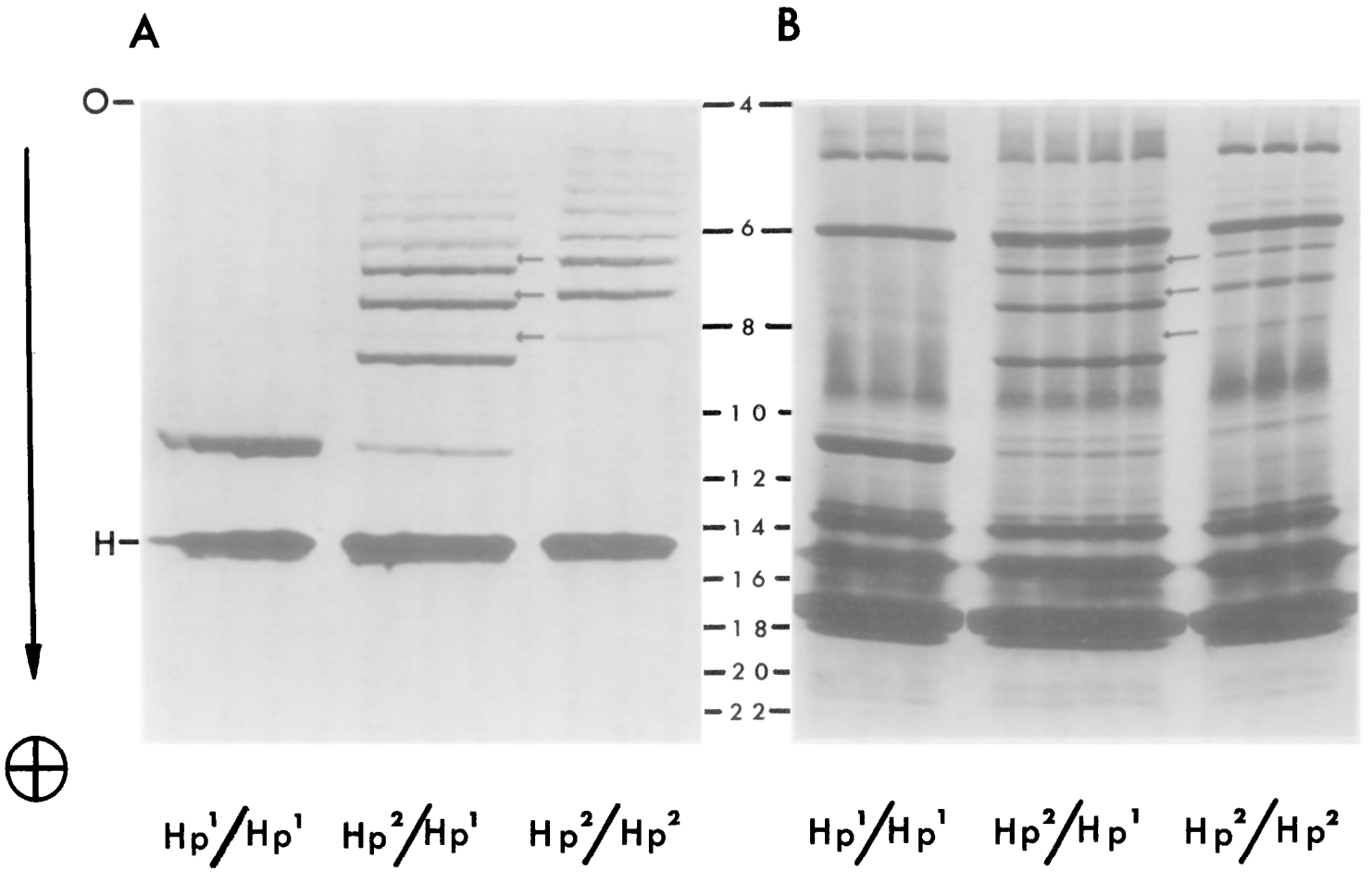

Figure 1. Separation of sera of Hp^1/Hp^1, Hp^2/Hp^1, and Hp^2/Hp^2 genotypes by gradient gel electrophoresis. A, benzidine stained gel showing haptoglobin/hemoglobin complexes and free hemoglobin (H); B, amido black stained gel. Arrows indicate Hp2-2 terms in the serum of the Hp^2/Hp^1 individual. Numbers in the column between A and B indicate acrylamide concentration per cent. O, origin; migration is towards the anode.

1-1 or 2-2 haptoglobin types. The actual ratio of 2-2 to 2-1 series appears to vary slightly from serum to serum.

Giblett (1964) has shown that the rare Carlberg Haptoglobin in which diminished production of α^1 chains is thought to occur, consists mainly of 2-2 components plus small amounts of the two fastest moving bands of the 2-1 series. Our work in conjunction with these findings, supports the contention that quantitative differences in expressivity of the Hp^1 and Hp^2 genes may result in variability of the Hp2-1, Hp2-2 ratio, in addition to ratio variations between terms of the 2-1 series.

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