Table 3. Scheme of LW.

Antigen	Anti-LW+LW_1	Incidence
LW_1	++	Rh+, ~ 85 percent
LW_2	+	Rh−, ~ 15 percent
lw	0	LW−, with or without Rh antigens (very rare)

(Table 1); anti-P disappeared and anti-M (*8*) was now evident.

It is significant that one absorption with LW-positive blood No. 4 was incomplete, but was nevertheless anti-LW specific, whereas one absorption with LW-negative blood No. 7 was complete. The apparent 85-percent specificity observed by Landsteiner and Wiener (*4*) and us (*5*) is attributable to the several treatments with Rh-negative blood required for complete absorption. If the absorption with Rh-negative blood were repeated until it became negative for itself and other Rh-negative bloods—the routine procedure carried out before our knowledge of LW specificity—the resulting supernatant would give reactions specific for random Rh-positive bloods. With this in view, repeated absorption experiments were carried out on two types of guinea pig antiserums to LW, with random Rh-positive and Rh-negative red cells. The absorptions were carried out as described for experiments listed in Table 1, except that one volume of washed, packed cells was used (Table 2).

In guinea pig antiserum to Rh-positive red cells, the high-incidence anti-LW specificity is still evident after two absorptions. A third absorption with Rh-positive blood removes almost all activity, whereas third and fourth absorptions with Rh-negative blood show 85 percent specificity.

In the guinea pig antiserum to baboon erythrocytes, one absorption with either Rh-positive or Rh-negative blood cells revealed anti-LW specificity. After the second, third, and fourth absorptions with both Rh-positive and Rh-negative blood, 85 percent specificity is observed, thus confirming Landsteiner and Wiener's original findings (*4*). Absorption with Rh-negative blood results in stronger reactions than absorption with Rh-positive blood cells.

In all instances in which anti-LW specificity is evident, reactions are stronger with Rh-positive than with Rh-negative blood, as in the case of human anti-LW serums (*1, 2*). In general, the loss of reactivity is greater on repeated absorption with Rh-positive blood. These observations suggest that Rh-positive red cells may have more LW sites than Rh-negative red cells.

The findings presented indicate that the antibody for the high-incidence LW factor is readily revealed in immunized guinea pigs and rabbits if their serums are properly absorbed and tested with a panel of red cells, including the rare LW-negative cells. The LW system shows remarkable parallels with A_1A_2O and P_1P_2p systems.

Thus anti-LW acts on LW_1 (about 85 percent Rh-positive) and LW_2 (about 15 percent Rh-negative). Anti-LW fails to react with the very rare LW negatives which may be called lw. Just as anti-A reacts more strongly with A_1 than A_2, so anti-LW reacts more strongly with LW_1 than with LW_2. Also absorption of anti-LW with LW_2 leaves behind a fraction active for LW_1. Thus anti-LW may be represented as anti-LW+LW_1. The analogies extend also to the P_1P_2p system (*9*).

Schematically these facts may be represented as shown in Table 3, insofar as direct reactions are concerned.

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References and Notes

1. P. Levine, M. J. Celano, J. Wallace, R. Sanger, *Nature* **198**, 596 (1963).
2. J. Swanson and A. G. Matson, *Transfusion* **4**, 257 (1964).
3. G. H. Vos, D. Vos, R. E. Kirk, R. Sanger, *Lancet* **1961-I**, 14 (1961); P. Levine, M. J. Celano, F. Falkowski, J. W. Chambers, O. B. Hunter, Jr., C. T. English, *Nature* **204**, 892 (1964); T. Ishimori and H. Hasekura, *Proc. Jap. Acad.* **42**, 658 (1966).
4. K. Landsteiner and A. S. Wiener, *J. Exp. Med.* **74**, 309 (1941).
5. P. Levine, M. J. Celano, R. Fenichel, W. Pollack, H. Singher, *J. Immunol.* **87**, 747 (1961).
6. P. Levine and M. J. Celano, *Nature* **193**, 184 (1962).
7. P. Levine, M. J. Celano, J. M. Staveley, *Vox Sang.* **3**, 434 (1958).
8. K. Landsteiner and A. S. Wiener, *J. Immunol.* **33**, 19 (1937).
9. R. R. Race and R. Sanger, *Blood Groups in Man* (Blackwell, Oxford, ed. 4, 1962), p. 126.

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Ring D Chromosome: A Second Case Associated with Anomalous Haptoglobin Inheritance

Abstract. *A second child with a ring D chromosome and anomalous inheritance of haptoglobin has been identified. Autoradiographic studies of peripheral lymphocytes from this child and of those from the previously described patient indicate that the ring in each is derived from chromosome No. 13. These findings are evidence that the locus for the haptoglobin alpha-chain is situated on one end of chromosome No. 13.*

A mentally retarded patient with multiple minor congenital anomalies and anomalous inheritance of haptoglobin has been described by Gerald *et al.* (*1*). Cytogenetic studies of that child revealed 46 chromosomes with a ring chromosome replacing one of the members of the D (13 to 15) group. The ring chromosome was presumably formed by breaks occurring at both ends of the D chromosome, with reunion of the broken ends and loss of the distal fragments. Typing of haptoglobin from family members indicated that the child had failed to inherit the paternal gene for haptoglobin. Since typing of the remaining blood group systems gave indirect confirmation of paternity, it was hypothesized that the paternal gene for haptoglobin was lost during formation of the ring and that the structural locus for the alpha-chain of haptoglobin (*2*) is situated on one end of a D chromosome.

A second and unrelated child with 46 chromosomes including a ring D, who also exhibits anomalous inheritance of haptoglobin, has now been studied. The clinical findings in this patient have been briefly reported (*3*). The results of haptoglobin typing in this second family are again most simply interpreted if it is assumed that the locus for the alpha-chain of haptoglobin is situated on one end of a D chromosome and that it has been lost during formation of the ring. We now report results of these studies and of the autoradiographic studies of both patients with ring D chromosomes.

Routine chromosomal analyses were performed by a modification of the method of Moorhead *et al.* (*4*). Studies of DNA replication with tritiated thymidine were done by a modification of previously described methods (*5, 6*). Typing of haptoglobin was performed by starch-gel electrophoresis of the hemoglobin-haptoglobin complexes. Subtyping of the alpha-chains of haptoglobin was done according to the method of Smithies (*7*). Quantitative determinations of haptoglobin were

performed according to the method of Tarukoski (*8*).

The patient described by Gerald *et al.* (*1*) is herein designated as case 1; that described by Reisman *et al.* (*3*), as case 2. Seventy-four cells from case 1 and 45 cells from case 2 were suitable for autoradiographic analysis. All cells from each patient lacked a normal No. 13 chromosome. This chromosome is identified as that member of the 13 to 15 group with late labeling primarily near the distal end of the long arm (*5*). [This same chromosome is that which is trisomic in the D_1 trisomy syndrome (*9*).] Both patients, therefore, have a ring derived from a No. 13 or D_1 chromosome. Since the haptoglobin studies of case 1 suggested that the locus for the alpha-chain of haptoglobin was lost during formation of the ring chromosome, similar studies were performed for case 2.

The results of the haptoglobin typing for the family of case 2 are given in Fig. 1. The father and several other relatives exhibited an unusual haptoglobin pattern, herein designated as 2-1WEAK (see Fig. 2) which resembles a 2-1 pattern except that the band closest to the anode is replaced by two weaker bands. In optimum electrophoretic runs each of the bands located closest to the cathode is duplicated as well (*10*). This unusual pattern is further characterized by a quantitative reduction in haptoglobin in individuals with the variant haptoglobin relative to that in normal family members (Fig. 1). On subtyping the haptoglobin from 2-1WEAK serums, we observed an alpha-chain band in the 2 position, but no band was observed in the 1 position (either in the "1^F" or the "1^S" position). The band in the 2 position was approximately equal in staining intensity to that found for a normal 2-1 individual; it was less intensely stained than that seen for a normal 2-2 individual. The amount of the 2 variety of alpha-chain was, therefore, consistent with the presence of a single 2 gene. The failure to demonstrate an allelic product was interpreted as evidence that the allelic gene produced very little product.

Typing of haptoglobin from the propositus' paternal grandfather shows a haptoglobin 1-1 pattern. The amount of haptoglobin present, however, is less than that customarily found in a normal 1-1 individual. When the haptoglobin is subtyped only a single band in the 1^S position is seen. From the nature of the haptoglobins of his progeny his genotype is presumed to be Hp^{1S}/Hp^{1WEAK}.

No haptoglobin could be detected in the serum of case 2, at ages 3½ and 4 years, even after a 15-fold concentration of his serum. This ahaptoglobinemia was not obviously attributable to adventitious disease—at least no evidence of hemolytic anemia or liver disease was found. No discrepancy in any other blood group system could be found. In view of the previous experience with case 1, the absence of haptoglobin in case 2 has been explained on the assumption that this child had a single gene for the haptoglobin alpha-chain.

The unusual electrophoretic pattern of haptoglobins from the father and three of his relatives indicates the presence of an atypical haptoglobin gene. This unusual pattern resembles the Carlberg phenotype described by

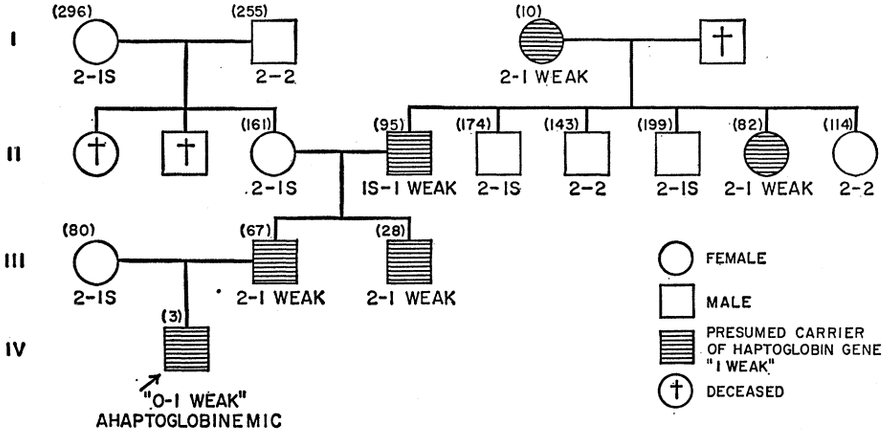

Fig. 1. Family pedigree of case 2. The figures under each symbol denote the assumed haptoglobin genotypes. The numbers in parentheses are the milligrams of haptoglobin per 100 ml of serum. The propositus is phenotypically "ahaptoglobinemic" but is presumed to have only the single haptoglobin gene *1WEAK*.

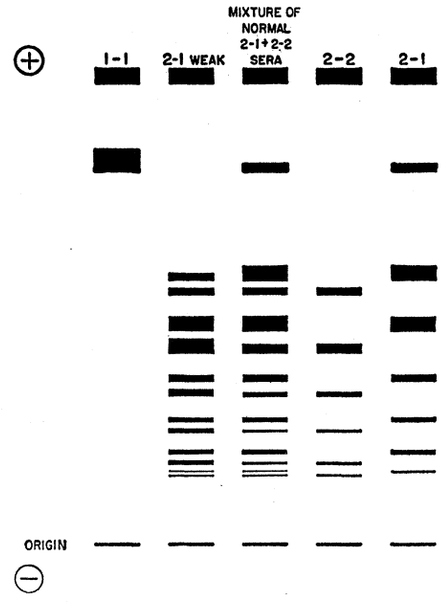

Fig. 2. Schematic representation of electrophoretic patterns of hemoglobin-haptoglobin complexes. From left to right: Hp 1-1, Hp 2-1WEAK (father of propositus), mixture containing 50 percent Hp 2-1 and 50 percent Hp 2-2 serums, Hp 2-2, and Hp 2-1. The fastest moving zone in each pattern is hemoglobin; all other bands are complexes of hemoglobin and haptoglobin.

Galatius-Jensen (*11*). Giblett has subtyped the Carlberg haptoglobin and has found a normal band in the 2 alpha-chain position but an abnormally weak band in the 1^S position (*12*). Giblett hypothesized that a variant of the 1 gene was present, which was characterized by a subnormal production of its corresponding chain. This interpretation of the Carlberg pattern has been confirmed by Bernini (*13*) who showed that the Carlberg phenotype could be simulated by recombination in vitro of isolated 1 alpha- and 2 alpha-chains in the ratio of nine parts of 2 to one part of 1.

This conclusion is also supported by studies of normal 2-1 and 2-2 haptoglobins. If the serums from individuals with these two normal haptoglobin types are mixed (Fig. 2), then an electrophoretic pattern like that of the unusual haptoglobin in the family of case 2 is obtained. These patterns are believed to result when there is an excess of haptoglobin 2 molecules, which can form polymers lacking any 1 molecules, in addition to forming the expected polymers containing both 2 and 1 molecules. On this basis, the variant 1 gene in the family of case 2 would be predicted to produce little product. Our failure to demonstrate the 1 component upon subtyping the

2-1WEAK serums is thought to result from the very small amount of this material present in this particular phenotype.

These various observations all support the contention that the haptoglobin genotypes of the parents of case 2 are Hp^2/Hp^{1WEAK} (father) and Hp^2/Hp^{1S} (mother). Their child with the ring 13 chromosome is believed to have inherited only the father's Hp^{1WEAK} gene. The expected maternal gene was presumably lost during formation of the ring chromosome. Our failure to detect haptoglobin in the serum of case 2 is believed to be consequent to the very limited amount of protein produced by the lone Hp^{1WEAK} gene.

It was previously pointed out that a silent gene in the family of case 1, rather than loss of genetic material during the formation of a ring chromosome, could conceivably have accounted for the anomalous inheritance of haptoglobin in this family (*1*). This was thought unlikely, however, in view of the infrequent occurrence of such a silent gene among Caucasians. A weak gene of the type we postulate for the family of case 2 could not have accounted for the findings on case 1 since the haptoglobin patterns associated with such "weak" genes (Carlberg or 2-1 modified) were not found.

These cases are the only two instances known to us of ring D chromosomes for which haptoglobin studies have been performed and for which autoradiographic analysis has identified the ring D chromosome specifically as chromosome No. 13. Both have been accompanied by anomalous inheritance of haptoglobin type. In each instance the findings may be explained if it is assumed that the locus determining the structure of the haptoglobin alpha-chain is lost during formation of the ring, and hence, that this locus is normally situated on one end or the other of chromosome No. 13.

There have been a limited number of studies of haptoglobin reported for families with other D-chromosome anomalies. De Grouchy *et al.* (*14*) reported the transmission of a D chromosome with a deletion of the short arm from an ahaptoglobinemic father to a son who was heterozygous for haptoglobin. No autoradiographic studies were reported, and, thus, the specific D chromosome involved is not known. If the ahaptoglobinemic state is related to the chromosomal defect in this family, this fact would indicate that a regulatory gene is involved rather than a structural one such as postulated for the two families we have studied.

Hustinx *et al.* (*15*) performed a limited analysis of linkage on families with D/G and D/D translocations and claimed a 14 percent recombination frequency of haptoglobin and the abnormal chromosome. They suggested that the haptoglobin locus is within mappable distance of the centromere in a D chromosome, although in a subsequent report they were unable to show any evidence for linkage after more detailed analysis (*16*). We have performed linkage calculations on the pedigrees of Hustinx *et al.* and on four of our own (two D/G; two D/D); the evidence is inadequate either to confirm or exclude linkage of the haptoglobin locus with the centromere (*17*). None of the families studied by Hustinx *et al.* and only one of ours was analyzed autoradiographically. In the absence of such information the interpretation of this linkage data is questionable.

It is quite probable that the structural locus for the haptoglobin alpha-chain is located on one end of chromosome No. 13; it is not possible to state with certainty whether this locus is on the end of the long arm or the short arm of this chromosome.

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References and Notes

1. P. S. Gerald, S. Warner, J. D. Singer, P. A. Corcoran, I. Umansky, *J. Ped.* **70**, 172 (1967).
2. Haptoglobin is a serum protein that exhibits a genetically determined variation. The genes commonly responsible for this variation are known as Hp^{1F}, Hp^{1S}, and Hp^2. These give rise to the phenotypes 1^F-1^F, 1^F-1^S, 1^S-1^S, 2-2, 2-1^F and 2-1^S. The haptoglobin molecule may be dissociated into subunits during urea-starch gel electrophoresis (a technique known as subtyping). The structural variation associated with this genetic polymorphism has been demonstrated by this means to reside within the alpha-chain subunit.
3. L. E. Reisman, A. Darnell, J. W. Murphy, *Lancet* **1965-II**, 445 (1965).
4. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. M. Hungerford, *Exp. Cell Res.* **20**, 613 (1960).
5. W. Schmid, *Cytogenetics* **2**, 175 (1963).
6. J. German, *J. Cell Biol.* **20**, 37 (1964).
7. O. Smithies, G. E. Connell, G. H. Dixon, *Amer. J. Hum. Genet.* **14**, 14 (1962).
8. P. H. Tarukoski, *Scand. J. Clin. Lab. Invest.* **18**, 80 (1966).
9. J. J. Yunis, E. B. Hook, M. Mayer, *Lancet* **1964-II**, 935 (1964).
10. For the reasons given in this report, the variant gene in this family is believed to be responsible for the production of very little product and hence is referred to as the *1WEAK* gene. Although the haptoglobin pattern in the father of the propositus closely resembles the previously described Carlberg phenotype, it is referred to as a Hp2-1WEAK pattern in this report so as to maintain consistency with the principles of nomenclature used in conjunction with abnormal hemoglobins. In the absence of a specific identification, such as an amino acid substitution, the descriptive terminology 2-1WEAK is preferred. Furthermore, results of subtyping the haptoglobins in this family are slightly different from those on the Carlberg phenotype, so the two may not be identical.
11. F. Galatius-Jensen, *Acta Genet.* **8**, 248 (1958).
12. E. R. Giblett, *Cold Spring Harbor Symp. Quant. Biol.* **24**, 321 (1964).
13. L. Bernini, C. Borri-Voltatorni, M. Siniscalco, *Proc. Int. Congr. Hum. Genet. 3rd*, 5–10 September (1966).
14. J. De Grouchy, Ch. Salmon, D. Salmon, P. Maroteaux, *Ann. Genet.* **9**, 80 (1966).
15. T. W. J. Hustinx, J. B. Bijlsma, L. E. Nijenhuis, *Nature* **207**, 328 (1965).
16. T. W. J. Hustinx, *Cytogenetisch Onderzoek Bij Enige Families* (Brakkenstein, Nijmegen, 1966), pp. 95–99.
17. G. E. Bloom and P. S. Gerald, unpublished data.
18. Supported by NIH grants HE 04706 and HE 5255 and by the John A. Hartford Foundation. Blood grouping studies were done by The Blood Grouping Laboratory, Boston, Massachusetts. We thank Dr. Louis K. Diamond for his helpful suggestions, Dr. Chester Alper for quantitative haptoglobin studies, and William Bucknall for assistance with the linkage calculations.

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Cholinesterase in Plasma: First Reported Absence in the Bantu; Half-life Determination

Abstract. *A Bantu schoolgirl is the first non-Caucasoid found homozygous for the "silent" cholinesterase gene. Investigation of her family revealed two heterozygotes. The propositus possesses no antibody to normal cholinesterase. Transfused cholinesterase had in her a half-life of approximately 10 days.*

The biologic importance of plasma cholinesterase (*Enzyme Nomenclature*: acylcholine acyl-hydrolase, 3.1.1.8) (ACAH) is not well understood, and subjects either completely lacking it or possessing only an atypical form are, under natural conditions, apparently perfectly healthy. However, ACAH is essential for the rapid breakdown of suxamethonium (succinylcholine), a drug frequently used by anesthetists, and, if the normal enzyme is completely absent or replaced by a variant, paralysis and prolonged apnoea, with serious and sometimes fatal consequences, may follow administration of the drug.

The most common variant of the enzyme is recognized by its lower sus-