percent in 20 groups when the D and G designations are used. A further increase to 98 percent correct assignments into 17 groups is achieved by pooling chromosome 4 with 5, 7 with X, and 10 with 12. These are initial measures of performance and they should not be generalized because the sample sizes are small, the data are from only one individual, and the learning and test set are drawn from the same analyses.

The means and standard deviations of the data from BHM are given in Table 1. Over this entire data set, the coefficient of variation of replicate measurements of DNA content is 1.5 percent, whereas homolog variability is 6 percent and metaphase variability is 3 percent. These values can be compared with the corresponding values for area measurements, which for these chromosomes are 2.4, 19, and 9 percent, respectively. Thus, when CYDAC is used with controlled focus, measurements of area are highly reproducible but show enormous homolog and metaphase effects caused by variations in compaction between homologs within the same cell and among corresponding chromosomes in different metaphases. The disparity between the behavior of DNA content and the behavior of area is even greater when the normalization by autosome totals is eliminated. For nonnormalized data, the metaphase variability for DNA content increases to 7 percent, and quadratic discrimination still correctly assigns 91 percent of BHM's chromosomes into 20 groups. However, for nonnormalized measurements of area, metaphase variability rises to 29 percent, and any attempt to do more than the crudest classification becomes a futile exercise.

Figure 3 shows the 50 percent tolerance regions for DNA content and centromeric index as calculated from the means and standard deviations given in Table 1. This diagram demonstrates the essential properties of the human chromosome set in terms of these two variables, and it illustrates both the readily discriminable and the unresolvable regions insofar as they have been determined for this one individual.

These initial results indicate the tractability, stability, and diagnostic potential of DNA-based variables of human chromosomes. Karyotyping with these variables far exceeds the resolution achieved with methods that were conventional before banding, and our method approaches the ultimate resolution now achieved with banding techniques. In a sense, DNA-based analysis actually exceeds the resolution of other methods, because we have detected differences between men and between homologs and these differences have no known counterpart in either conventional or banding techniques. This resolution will very likely provide useful markers for linkage studies. The corresponding resolution in the absence of normalization also makes our methods uniquely suited to the analysis of hybrid and aneuploid cells. Finally, DNA content, although of major significance in its own right, is only one example of the potential set of photometrically exploitable biochemical characteristics of the chromosome.

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Chromosome Assignments in Man of the Genes for Two Hexosephosphate Isomerases

Abstract. *Thirty-seven clones of somatic cell hybrids between human and mouse cells were examined for retention of human chromosomes and expression of human constitutive enzymes. Human glucosephosphate isomerase and chromosome F-19 were retained or lost concordantly, as were human mannosephosphate isomerase and chromosome C-7. The genes for the enzymes are thus assigned to these two chromosomes.*

Somatic cell hybrids can be useful in human genetics, particularly in establishing the linkage (or synteny) of human enzymes to each other and to individual human chromosomes. Somatic cell hybrids between human and mouse cells can be obtained in tissue culture by cell fusion mediated by Sendai virus (*1*), and the hybrids isolated from the parents by drug selection (*2, 3*). The human-mouse hybrids so obtained preferentially segregate human chromosomes (*4*); thus, analysis of the complement of human chromosomes and human constitutive enzymes retained by numerous independently derived hybrid clones leads to the assignment of the genes for those enzymes to the chromosomes bearing them (*5*). By this means, we have assigned the human gene for glucosephosphate isomerase (GPI) (E.C. 5.3.1.9) [phosphohexose isomerase, 17240 (*6*)] to the F-19 chromosome, and that for mannosephosphate isomerase (MPI) (E.C. 5.3.1.8) to the C-7 chromosome. These results, referred to previously (*5*), are presented here in complete form. Linkage of the human gene for GPI to chromosome F-19 has been confirmed by Hamerton *et al.* (*7*).

Six different series of human-mouse hybrids were studied. The IL hybrids were produced by fusing human neuroblastoma cells of strain IMR-32 with mouse L cells of strain LM(TK$^-$), deficient in thymidine kinase (E.C. 2.7.1.21). The NM hybrids were obtained by fusing human skin fibroblasts (MRC-5) and the mouse neuroblastoma cell clone NA, which is deficient for hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (E.C. 2.4.2.8). The IL and NM hybrids will be described in detail elsewhere (*8*). The RK hybrids (*9*) were derived by fusing hu-

Table 1. Concordant segregation of glucosephosphate isomerase (GPI) with the F-19 chromosome and of mannosephosphate isomerase (MPI) with the C-7 chromosome. Data for GPI are taken from 1078 cells of 37 hybrid clones of series IL (4 clones), NM (1 clone), WA (13 clones), JBA (1 clone), RK (10 clones), and J (8 clones). Clones with the karyotype 2s (mouse) + 1s (human) are omitted from the MPI tabulation, which represents 678 cells (19 clones) of series IL (4 clones), NM (1 clone), WA (9 clones), and J (5 clones). Different numbers of clones were used in the GPI and MPI correlations because (i) we have assayed for GPI for a longer period than for MPI and (ii) 2s clones were eliminated in the MPI series, as described in the text.

Enzyme/ chromosome	Number of clones			
	+/+	+/−	−/+	−/−
GPI/F-19	9	0	0	28
MPI/C-7	7	0	0	12

man skin fibroblasts of strains KOP-1 or KOP-2, bearing a reciprocal translocation between chromosomes D-14 and X, with the HGPRT$^-$ mouse RAG cell line (*1*). The translocation is not relevant to this study. The J series were obtained by fusing human peripheral leukocytes with RAG. The WA and JBA series were derived by fusing a mouse L cell line deficient for adenine phosphoribosyltransferase (APRT) (E.C. 2.4.2.7) with human lung fibroblasts (WI-38) or human peripheral leukocytes (JB leukocytes), respectively.

In all cases, cell fusion was mediated by Sendai virus killed with β-propiolactone, essentially the method of Klebe *et al.* (*1*), either in monolayer culture or, in the case of leukocyte fusions, in suspension. Hybrids were selected from the parents either by the hypoxanthine-aminopterin-thymidine (HAT) system of Littlefield (*2*) or by a modification (*10*) of the alanosine-adenine system of Kusano *et al.* (*3*). The HAT medium was made by supplementing the usual tissue culture medium (the Dulbecco-Vogt modification of Eagle's medium, supplemented with 10 percent fetal calf serum or newborn calf serum free of gamma globulin) with $10^{-4}M$ hypoxanthine, 4×10^{-7} aminopterin, and $1.6 \times 10^{-5}M$ thymidine. Cells deficient for thymidine kinase or HGPRT are unable to grow in this medium because endogenous purine and pyrimidine synthesis is inhibited by aminopterin and utilization of the exogenous sources is prevented by the enzyme deficiencies. Hybrids contain both thymidine kinase and HGPRT by complementation, and grow as clones in the culture vessels. In hybridizations in which one parent was deficient for APRT, the alanosine-adenine selective medium was used. This medium was supplemented with alanosine (7 μg/ml) (the gift of P. Sensi) and $5 \times 10^{-5}M$ adenine. The basis of selection is similar to that in HAT medium. Alanosine inhibits endogenous adenylate synthesis, and APRT is necessary to utilize the exogenous adenine; hence, APRT-deficient parents die but hybrids survive.

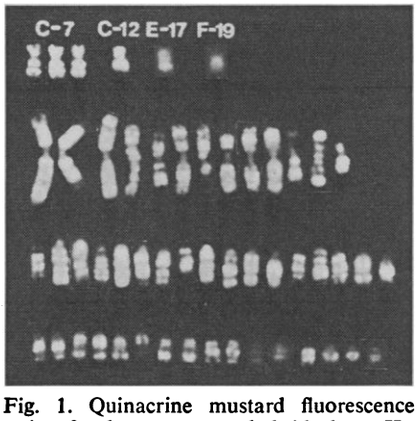

Fig. 1. Quinacrine mustard fluorescence stain of a human-mouse hybrid clone, IL-II-5, which expressed the human forms of glucosephosphate isomerase and mannosephosphate isomerase. Human chromosomes are labeled; others are derived from the LM(TK$^-$) mouse parent.

Hybrid clones were analyzed by starch-gel electrophoresis for the presence of the human and mouse forms of GPI (*11*) and MPI (*12*). The following 21 enzymes were also assayed by starch-gel or acrylamide-gel electrophoresis (*10, 13*): adenosine deaminase (E.C. 3.5.4.4); APRT; glutamate-oxaloacetate transaminase (E.C. 2.6.1.1); glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49); HGPRT; isocitrate dehydrogenase (E.C. 1.1.1.42); indolephenol oxidase A, tetrameric form; indolephenol oxidase B, dimeric form; lactate dehydrogenase A (E.C. 1.1.1.27); lactate dehydrogenase B (E.C. 1.1.1.27); malate oxidoreductase (decarboxylating) (E.C. 1.1.1.40); malate oxidoreductase (E.C. 1.1.1.37); nucleoside phosphorylase (E.C. 2.4.2.1); peptidase A; peptidase B; peptidase C; peptidase D; phosphoglycerate kinase (E.C. 2.7.2.3); phosphoglucomutase 1 (E.C. 2.7.5.1); and thymidine kinase. For chromosomal analysis, air-dried preparations of Colcemid-arrested metaphase cells were made by routine methods and stained by four methods: (i) 1.5 percent aceto-orcein, (ii) centromeric constitutive heterochromatin (*14*), (iii) Giemsa banding (*15*), and (iv) quinacrine mustard fluorescence (*16*). Individual human chromosomes were identified (*17*) in karyotypes constructed from photomicrographs of metaphase spreads. A hybrid cell stained by the quinacrine mustard fluorescence method is shown in Fig. 1.

In the 37 primary and secondary clones examined, the human F-19 chromosome and the human form of GPI were either retained together or lost together. Presence of this human enzyme in a clone was accompanied by the presence of chromosome F-19 in 13 to 70 percent of the cells examined. Human GPI activity could not be detected in two clones in which F-19 chromosomes were present in 6 percent of cells or less. Since the activity of human GPI present in these hybrids would be below the limit of detectability in assays of mixtures of cell ex-

Table 2. Correlation of human enzyme expression with the presence of human chromosomes. The + columns give the number of clones in which each human enzyme and chromosome are present together or absent together (concordant segregation); the − columns give the number of clones in which only the enzyme or the chromosome is present (discordant segregation). Asterisks in the nonlinkage (NL) columns indicate that additional data support nonlinkage of GPI or MPI to another enzyme known to be linked to that chromosome. The data show nonlinkage of GPI to all chromosomes except F-19 and nonlinkage of MPI to all chromosomes except C-7. Clones included are from the J, WA, JBA, IL, and NM series, with clones of the karyotype 2s (mouse) + 1s (human) omitted from the MPI tabulation.

	GPI			MPI		
	+	−	NL	+	−	NL
A-1	13	12	*	19	1	*
A-2	20	5		13	7	
A-3	15	10		16	4	
B-4	17	8		10	10	
B-5	22	3		14	6	
C-6	15	10	*	15	5	*
C-7	15	10	*	20	0	
C-8	16	9		13	7	
C-9	21	4		13	7	
C-10	11	14	*	16	4	*
C-11	15	10	*	17	3	*
C-12	15	10	*	15	3	*
X	13	12	*	18	2	*
D-13	17	8		10	10	
D-14	19	6	*	11	9	*
D-15	20	5		14	6	
E-16	11	14	*	12	8	*
E-17	12	13	*	14	6	*
E-18	10	15	*	16	4	*
F-19	25	0		14	6	*
F-20	18	7		10	10	
G-21	14	11	*	13	7	*
G-22	17	8		9	11	
Y	21	4		13	7	

tracts (*18*), these two clones were considered to be lacking both GPI and the F-19 chromosome. By these criteria, nine clones were positive for both GPI and F-19, 28 clones were negative for both, and no clones were positive for one and negative for the other (Table 1). The presence of human GPI did not correlate with the presence of any other human chromosome (Table 2).

Glucosephosphate isomerase is loosely linked to the β chain of hemoglobin in the mouse (*6*). Since variants of GPI and of the β chain are present in humans, this linkage could be investigated in man by classical family studies. The β and δ chains of hemoglobin are very closely linked in man (*6*). If GPI and the β chain are linked in man, this would place the genes for GPI and the β and δ chains on the human F-19 chromosome.

Concordant segregation of human MPI and chromosome C-7 was also observed in most cases (Tables 1 and 2). When hybrid clones containing one mouse chromosome set plus one reduced human set (1s + 1s) were analyzed, MPI and C-7 were always present together or absent together (Table 1). Presence of human MPI did not correlate with the presence of any human chromosome other than C-7 (Table 2). When subclones were derived from one clone that expressed GPI and MPI, one subclone obtained (WAIaD6) had lost two human chromosomes, C-7 and F-19, and had also lost both enzyme phenotypes. These data support the linkage of the human gene for MPI to the C-7 chromosome.

Data for hybrid clones containing two mouse chromosome sets and one reduced human chromosome set (2s + 1s) failed to support the linkage of MPI to C-7. Five clones of this makeup contained C-7 chromosomes but did not express human MPI. Four of these five clones were derived from the RK hybrid series. Data for these same clones failed to support two other human gene-chromosome linkage relationships; in each case, the chromosome was present but the enzyme was not expressed. A subclone of one of these clones reexpressed a human enzyme that was anomalously unexpressed in the parental hybrid clone. These considerations suggest that some phenomenon in hybrids with the karyotype 2s (mouse) + 1s (human) may interfere with the expression of enzymes generally considered to be constitutively expressed (*19*). The data from 1s + 1s hybrids (Tables 1 and 2) consistently support the linkage of human MPI to chromosome C-7 and make the linkage of MPI to any other chromosome unlikely. Linkage of MPI to C-7 is therefore indicated, but is not as firmly established as the linkage of GPI to F-19.

We have reported the linkage of lactate dehydrogenase subunits A and B, which are very closely related in structure and function, to the morphologically similar human chromosomes C-11 and C-12, respectively (*20*). It has been suggested that chromosome duplication at some point in evolution could have provided the genetic raw material for producing new, but similar, chromosomes and polypeptide sequences (*21*). In the present case, two hexosephosphate isomerases have been linked to chromosomes that are at least superficially dissimilar (Fig. 1). Structural dissimilarity is also suggested for the enzymes; GPI is a dimer (*11*), whereas the electrophoretic pattern of MPI from hybrid mice and from interspecific somatic cell hybrids suggests that MPI is a monomer (*12*). Further research in somatic cell genetics may thus contribute to our understanding of molecular evolution as well as of human genetics.

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Cholesterol Hydroperoxide Formation in Red Cell Membranes and Photohemolysis in Erythropoietic Protoporphyria

Abstract. *3β-Hydroxy-5α-hydroperoxy-Δ⁶-cholestene is produced in protoporphyrin-containing red blood cell ghosts irradiated with approximately 400-nanometer light in the presence of oxygen. Incorporation of this cholesterol photooxidation product into normal red blood cells leads to increased osmotic fragility and eventual hemolysis. These results may be relevant to photohemolysis associated with erythropoietic protoporphyria.*

The red blood cells (RBC) of patients possessing the genetic disorder erythropoietic protoporphyria (EPP) (*1*) contain a much larger amount of free porphyrin than is found in normal RBC (*2*). These porphyrinemic RBC undergo facile hemolysis upon irradiation in vitro with visible light, whereas normal cells do not (*3*). Although there is no direct evidence that photohemolysis is the basis for the severe cutaneous photosensitivity of EPP patients, the phenomenon has received much attention (*4–7*), largely because (i) in vitro photohemolysis serves as a useful diagnostic criterion for EPP (*8*); (ii) photohemolysis may be a useful model for the skin sensitivity; and (iii) photohemolysis is associated with damage to the RBC membrane, the structure of which is being widely studied.

Wavelengths of light near 400 nm are the most effective for both the skin effects and photohemolysis (*1–4*). This