in erythrocytes and not in fibroblasts or in lymphoblasts (*7, 24*).

Whether the increased enzyme activity observed in our patients results from a structural alteration in the PRPP synthetase molecule or from an increase in amount of this enzyme in the cells remains to be determined. Identical patterns of inactivation of PRPP synthetase at 60°C were found in hemolyzates of normal subjects and of patient H.B. Measurement of affinity constants for ATP and ribose 5-phosphate in hemolyzates have shown no difference between normal and patient's enzyme for either substrate. The sensitivity of the patient's enzyme to inhibition by purine nucleotides does not appear altered from normal in studies with adenosine diphosphate or guanosine diphosphate as inhibitors.

The increased PRPP synthetase activity in these patients' cells is apparent at all concentrations of added inorganic phosphate (Fig. 1). This differentiates the abnormality in PRPP synthetase activity of our patients from that of the patient of Sperling *et al.* (*25*), in which increased enzyme activity was identifiable only at concentrations of inorganic phosphate up to 2 m*M*. The existence of at least two separate abnormalities in PRPP synthetase activity associated with purine overproduction implies that a range of separable defects in this activity may exist among patients with clinical gout, much as a variety of abnormalities in HGPRT have been discovered among patients with the Lesch-Nyhan syndrome (*26*).

Our studies, relating an increased enzyme activity with increased intracellular production of a regulatory substrate and concomitant overactivity of an entire pathway, demonstrate that disease states in man may result from genetic alterations leading to increased as well as diminished enzyme function. Increased activity of hepatic δ-aminolevulinic acid synthetase in acute intermittent porphyria has been postulated to represent an important factor in the pathogenesis of that disease (*27*). Studies of Strand *et al.* (*28*) indicate, however, that the increased activity of δ-aminolevulinic acid synthetase is secondary to a partial deficiency of uroporphyrinogen I synthetase, with resulting alterations in the intracellular concentration of negative feedback effectors or inducers of δ-aminolevulinic acid synthetase. The possibility of a similar mechanism to account for increased PRPP synthetase activity in our patients' cells cannot yet be dismissed.

The mode of inheritance of the PRPP synthetase abnormality discussed here remains to be defined. Transmission of the abnormality from father (H.B.) to daughter (C.B.) without detectable abnormality in the mother's (Y.B.) enzyme activity suggests dominant inheritance that is either autosomal or X-linked.

MICHAEL A. BECKER
LAURENCE J. MEYER
ALEXANDER W. WOOD
J. EDWIN SEEGMILLER
Department of Medicine, University of California, San Diego, La Jolla 92037

References and Notes

1. J. E. Seegmiller, in *Duncan's Diseases of Metabolism*, P. K. Bondy, Ed. (Saunders, Philadelphia, ed. 6, 1969), pp. 517–541.
2. ———, F. M. Rosenbloom, W. N. Kelley, *Science* **155**, 1682 (1967); W. N. Kelley, F. M. Rosenbloom, J. E. Seegmiller, R. R. Howell, *J. Pediat.* **72**, 488 (1968).
3. F. M. Rosenbloom, J. F. Henderson, I. C. Caldwell, W. N. Kelley, J. E. Seegmiller, *J. Biol. Chem.* **243**, 1166 (1968).
4. T. F. Yü, M. E. Balis, T. A. Krenitsky, J. Dancis, D. N. Silvers, G. B. Elion, A. B. Gutman, *Ann. Int. Med.* **76**, 255 (1972).
5. I. H. Fox and W. N. Kelley, *ibid.* **74**, 424 (1971).
6. J. B. Wyngaarden and W. N. Kelley, in *The Metabolic Basis of Inherited Disease*, J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, Eds. (McGraw-Hill, New York, ed. 3, 1972), p. 911.
7. A. W. Wood, M. A. Becker, J. E. Seegmiller, *Biochem. Genet.*, in press. M. L. Greene and J. E. Seegmiller, *Arthritis Rheum.* **12**, 666 (1969).
8. I. H. Fox, J. B. Wyngaarden, W. N. Kelley, *N. Engl. J. Med.* **283**, 1177 (1970); J. D. Schulman, M. L. Greene, W. Y. Fujimoto, J. E. Seegmiller, *Pediat. Res.* **5**, 77 (1971); J. E. Seegmiller, J. R. Klinenberg, J. Miller, R. W. E. Watts, *J. Clin. Invest.* **47**, 1193 (1968); W. N. Kelley, M. L. Greene, I. H. Fox, F. M. Rosenbloom, R. I. Levy, J. E. Seegmiller, *Metabolism* **19**, 1025 (1970).
9. J. A. Boyle, K. O. Raivio, M. A. Becker, J. E. Seegmiller, *Biochim. Biophys. Acta* **269**, 179 (1972); W. N. Kelley, I. H. Fox, J. B. Wyngaarden, *ibid.* **215**, 5120 (1970).
10. J. E. Seegmiller, A. I. Grayzel, L. Laster, L. Liddle, *J. Clin. Invest.* **40**, 1304 (1961).
11. A. Hershko, A. Razin, J. Mager, *Biochim. Biophys. Acta* **184**, 64 (1969).
12. M. Lesch and W. L. Nyhan, *Amer. J. Med.* **36**, 561 (1964).
13. J. F. Henderson and M. K. Y. Khoo, *J. Biol. Chem.* **240**, 2349 (1965); M. L. Greene, J. A. Boyle, J. E. Seegmiller, in preparation.
14. J. F. Henderson, A. R. P. Paterson, I. C. Caldwell, M. Hori, *Cancer Res.* **27**, 715 (1967).
15. J. F. Henderson, F. M. Rosenbloom, W. N. Kelley, J. E. Seegmiller, *J. Clin. Invest.* **47**, 1511 (1968).
16. A. W. Wood and J. E. Seegmiller, *J. Biol. Chem.* **248**, 138 (1973).
17. M. Tatibana and K. Shigesada, *Advan. Enzyme Regul.* **10**, 249 (1972).
18. E. B. Thompson, G. M. Tomkins, J. F. Curran, *Proc. Nat. Acad. Sci. U.S.A.* **56**, 296 (1966).
19. R. T. Schimke, E. W. Sweeney, C. M. Berlin, *J. Biol. Chem.* **240**, 322 (1965).
20. M. L. Greene, J. A. Boyle, J. E. Seegmiller, *Science* **167**, 887 (1970).
21. A. Yoshida, *J. Mol. Biol.* **52**, 483 (1970); ——— and A. G. Motulsky, *Amer. J. Hum. Genet.* **21**, 486 (1969).
22. R. Dreisbach, *Anal. Biochem.* **10**, 169 (1965).
23. W. N. Valentine and K. K. Kürschner, *Blood* **39**, 666 (1972).
24. W. N. Kelley, *J. Lab. Clin. Med.* **77**, 33 (1971).
25. O. Sperling, P. Boer, S. Persky-Brosh, E. Kanarek, A. de Vries, *Eur. J. Clin. Biol. Res.* **17**, 703 (1972).
26. W. N. Kelley and J. C. Meade, *J. Biol. Chem.* **246**, 2953 (1971).
27. D. P. Tschudy, M. G. Perlroth, H. S. Marver, A. Collins, G. Hunter, M. Rechcigl, *Proc. Nat. Acad. Sci. U.S.A.* **53**, 841 (1965).
28. L. J. Strand, B. J. Felsher, A. G. Redeker, H. S. Marver, *ibid.* **67**, 1315 (1970).
29. G. W. Crabtree and J. F. Henderson, *Cancer Res.* **31**, 985 (1971).
30. Supported in part by grants AM 13622, AM 05646, and GM 17702 from the National Institutes of Health and by grants from the National Genetics Foundation and the San Diego County Heart Association.

18 September 1972; revised 4 December 1972 ■

DNA Content and DNA-Based Centromeric Index of the 24 Human Chromosomes

Abstract. *The chromosomes of two human males were identified by fluorescent banding, restained, and measured by scanning microscopy and computer analysis. The two variables, DNA content and DNA-based centromeric index, provided almost complete discrimination of chromosome types. Some chromosomes showed significant differences in DNA content between the men, and for one man two pairs of chromosomes showed significant differences between homologs.*

The fluorescent banding method of Caspersson *et al.* (*1*) allows every chromosome in selected human metaphase cells to be identified with assurance. After restaining such chromosomes with a DNA-specific procedure, we can measure their DNA content and DNA-based centromeric index using our techniques of quantitative image analysis. We present, in this first report on these combined methods, the chromosomal DNA contents from two normal human males and the centromeric index based on DNA content from six cells of one of the men.

Previously reported, generally standard, and newly developed procedures were combined into the following steps:

1) Blood samples from two adult males, BHM and DHM, who have no known genetic or medical disorder, were cultured and prepared for chromosome analysis by standard techniques (*2*).

2) The cells were stained with 0.0005 percent quinacrine hydrochloride and

then mounted in 10 percent neutral formalin to inhibit any interim digestion of the chromosomes.

3) Complete metaphases showing good fluorescent banding and a minimum of touching chromosomes were selected and photographed.

4) The selected cells were karyotyped by at least two observers working independently from prints of the fluorescent photographs. Assignment of chromosomes followed the system of Caspersson *et al.* (*1*).

5) The quinacrine was removed by washing first in water and then in absolute alcohol.

6) The cells were digested with ribonuclease and stained with gallocyanine-chrome alum (*3*).

7) Each metaphase was scanned and digitized directly with the Cytophotometric Data Converter (CYDAC), a high-resolution, flying-spot scanning cytophotometer (*4*), with the use of a recently developed focus-assist device to ensure optimum focus of the scanning microscope (*5*).

8) The digital images were analyzed automatically by computer. For each chromosome an optimum threshold was calculated and points above this threshold were identified as "core." A region extending 1 μm beyond the core was labeled "periphery." Background grayness was computed from composite grayness profiles (*6*); a local background was used for the periphery, and an iteratively computed diffuse background for the core. The content of DNA stain was the sum of optical densities of all points in core and periphery. The centromere was located by a new procedure using a boundary analysis similar to that of Gallus and Neurath (*7*) and the minimal strip optical density method (*8*).

9) The results were expressed as DNA content (that is, content of chromosomal DNA stain as a percentage of the total for autosomes in that cell) and centromeric index (that is, the ratio of DNA content of the large or long arm to total DNA content for that chromosome).

Table 1. Chromosomal measurements from six cells. The standard deviations (S.D.) include the effects of replication, homolog variability, and metaphase variability.

Chromosome	DNA content (% of autosome total*)		Centromeric index (large arm/total)	
	Mean	S.D.	Mean	S.D.
1	4.32	0.08	0.51	0.01
2	4.22	.14	.61	.02
3	3.49	.09	.53	.02
4	3.34	.10	.72	.02
5	3.20	.07	.73	.01
6	3.02	.06	.65	.03
7	2.77	.05	.61	.02
X	2.70	.05	.62	.01
8	2.55	.07	.67	.01
9	2.37	.05	.65	.02
10	2.33	.03	.69	.01
11	2.38	.06	.59	.02
12	2.35	.06	.72	.02
13	1.86	.12	.85	.01
14	1.80	.05	.85	.03
15	1.69	.06	.84	.03
16	1.55	.05	.60	.02
17	1.49	.06	.68	.04
18	1.40	.03	.75	.02
20	1.20	.03	.55	.02
19	1.08	.04	.55	.03
Y	0.92	.05	.76	.02
22	.86	.03	.77	.03
21	.82	.06	.76	.03
Summary	2.252†	0.068‡	0.677†	0.023‡

* In each cell, the autosome total is defined as the sum of the content of the DNA stain of the 44 chromosomes other than sex chromosomes. † Mean DNA content and centromeric index of the 46 male chromosomes. ‡ Summary standard deviations, based on weighted mean variances. Standard errors of the mean are approximately one-third of the values shown.

The DNA contents of the 24 chromosome types of the two normal men are shown in Fig. 1. The descending sequence by chromosome number that one would expect from the Denver convention is seen, except for (i) the reversal of chromosomes 19 and 20 and of 21 and 22; (ii) the position of chromosome X between 7 and 8; and (iii) the identical content of chromosomes 1 and 2; of 9, 10, 11, and 12; and of 17 and 18. In general the agreement between the two men is good, but there are significant differences for chromosomes 1, X, 16, and 21. For chromosomes 1, X, and 16, the respective differences in DNA content are 3.5 percent ($P < .01$), 2.6 percent ($P < .01$), and 3.2 percent ($P < .02$). We have found heterogeneity of DNA content of chromosome 1 in a previous case (*9*), and others have similar results with respect to morphology, length, and constitutive heterochromatin for both chromosomes 1 and 16 (*10*). The disparity for chromosome 21 is due entirely to one chromosome 21 homolog in DHM that is marked with a large brightly fluorescent satellite. This chromosome has a significantly greater DNA content than its homolog, an apparent excess of about 10 percent or 10^{-14} g of DNA. Also, the DNA contents of homologs of chromosome 2 in DHM differ by 4.5 percent ($P < .01$). None of the homolog pairs of BHM show significant internal differences.

As shown in Fig. 2, 251 chromosomes from six cells of BHM have been analyzed for both DNA content and centromeric index. The data group into the same broad clusters found for previous results (*11*) and for the length and area measurements by others (*12*). However, in addition to the 10 or 11 previously definable groups, there are also subclusters that correspond precisely with the identifications based on fluorescent banding. Thus, the improvements in image analysis combined with the independent identification of each chromosome by banding permit us to assign a unique cluster for all but the acrocentric chromosomes in the D group (chromosomes 13, 14, and 15) and G group (chromosomes 21, 22, and Y).

Machine classification of the chromosomes in Fig. 2 confirms the visual impression that the clustering is significant. A method of quadratic discrimination using the two variables, DNA content and centromeric index, correctly places 85 percent of the chromosomes into the 24 categories even when no attempt is made to modify assignments by pairing rules within each cell. Correct placement increases to 93

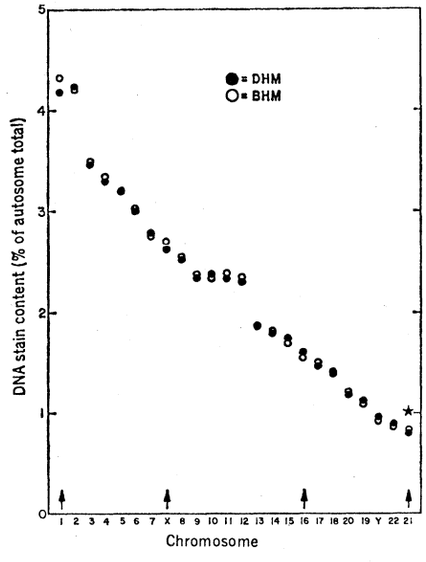

Fig. 1. DNA content of the 24 chromosome types of two normal males. For the autosomes (except chromosome 21 in DHM), means for the homolog pairs from cells are given for each subject. One chromosome 21 homolog for DHM (starred point) has a prominent fluorescent marker and is approximately 10 percent higher in DNA content than either its homolog or the two chromosome 21 homologs of BHM. The arrows mark chromosomes with significant differences between subjects. The chromosomes are ranked by DNA content or, in the cases of unresolvable differences, by the Denver convention. Note the unconventional rank of chromosome X compared to 7, 19 compared to 20, and 21 compared to 22.

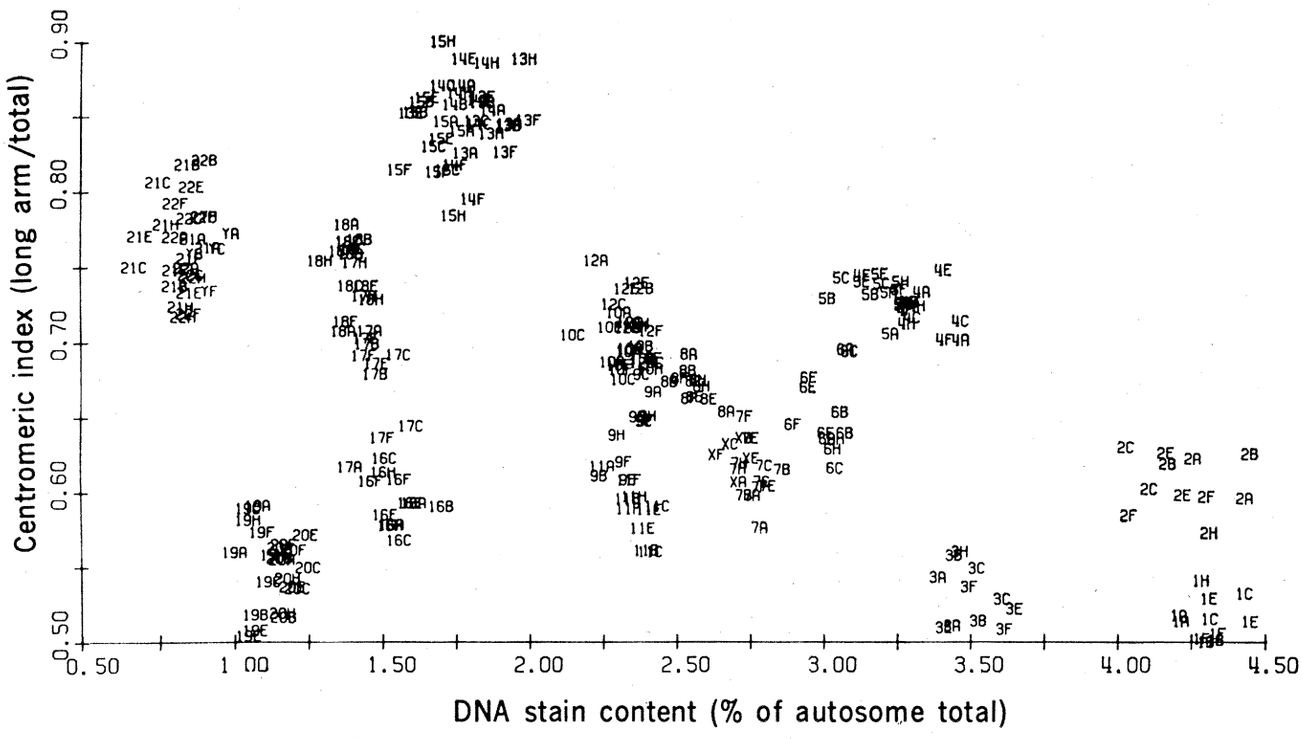

Fig. 2. DNA content and centromeric index for the chromosomes from six cells of BHM. The number and letter code correspond, respectively, to the fluorescent karyotype assignment and the cell from which the individual chromosome came. Of the 24 karyotype assignments, 13 compared to 14 and 21 compared to 22 are the only pairs whose means are not significantly different by Hotelling's T^2 test.

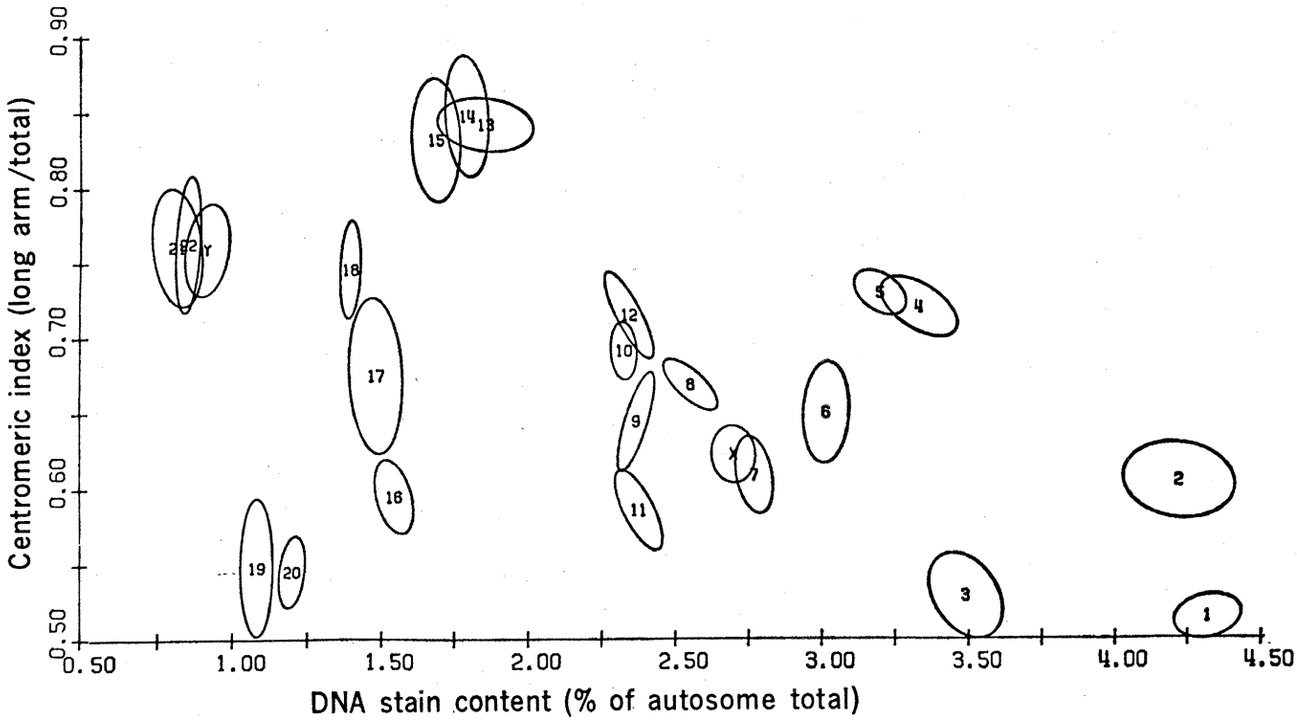

Fig. 3. The 50 percent tolerance regions of BHM's chromosomes. There is one region for each of the 24 chromosome types. The elliptical boundaries are calculated from the estimated variances and covariances, and the identifying numbers are centered on the means of DNA content and centromeric index of the corresponding chromosomes. In this fingerprint of the human karyotype, there are clear separations of many chromosomes, partial separations of the B group (chromosomes 4 and 5) and some of the C group (chromosome 7 compared to X and 10 compared to 12), and superimpositions within the D group (chromosomes 13, 14, and 15) and within the G group (chromosomes 21, 22, and Y).

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.

MORTIMER L. MENDELSOHN*
BRIAN H. MAYALL*, ELLIOT BOGART*
DAN H. MOORE II*, BENSON H. PERRY
Department of Radiology,
University of Pennsylvania,
Philadelphia 19104

References and Notes

1. T. Caspersson, G. Lomakka, L. Zech, *Hereditas* **67**, 89 (1971).
2. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. A. Hungerford, *Exp. Cell Res.* **20**, 613 (1960).
3. B. H. Mayall, *J. Histochem. Cytochem.* **17**, 249 (1969).
4. M. L. Mendelsohn, B. H. Mayall, J. M. S. Prewitt, R. C. Bostrom, W. G. Holcomb, *Advan. Opt. Electron Microsc.* **2**, 77 (1968).
5. M. A. Kujoory, B. H. Mayall, M. L. Mendelsohn, *IEEE Trans. Bio-Med. Eng.*, in press.
6. M. L. Mendelsohn, B. H. Mayall, B. H. Perry, in *Advances in Medical Physics*, J. S. Laughlin and E. W. Webster, Eds. (Second International Conference on Medical Physics, Boston, 1971), pp. 327–341.
7. G. Gallus and P. W. Neurath, *Phys. Med. Biol.* **15**, 435 (1970).
8. M. L. Mendelsohn, B. H. Mayall, J. M. S. Prewitt, in *Image Processing in Biological Science*, D. M. Ramsey, Ed. (Univ. of California Press, Berkeley, 1968), pp. 119–136; J. Hilditch and D. Rutovitz, *Ann. N.Y. Acad. Sci.* **157**, 339 (1969).
9. M. L. Mendelsohn, D. A. Hungerford, B. H. Mayall, B. H. Perry, T. J. Conway, J. M. S. Prewitt, *Ann N.Y. Acad. Sci.* **157**, 376 (1969).
10. J. Leisti, *Ann. Acad. Sci. Fenn. Ser. A4* **179**, 1 (1971); H. A. Lubs and F. H. Ruddle, *Nature* **233**, 134 (1971); A. P. Craig-Holmes and M. W. Shaw, *Science* **174**, 702 (1971).
11. M. L. Mendelsohn, T. J. Conway, D. A. Hungerford, W. A. Kolman, B. H. Perry, J. M. S. Prewitt, *Cytogenetics* **5**, 223 (1966).
12. K. Patau, *Amer. J. Human Genet.* **12**, 250 (1960); R. Turpin and J. Lejeune, *Les Chromosomes Humains* (Gauthier-Villars, Paris, 1965).
13. Supported by NIH grants 5 R01 GM16913 and K6 CA18,540. We acknowledge the technical assistance of V. Alpert, P. Dave, K. Lane, and J. Colsher.
* Present address: Bio-Medical Division, Lawrence Livermore Laboratory, University of California, Livermore 94550.

30 July 1972; revised 18 December 1972

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-