process brought about by covalent binding of platinum. We suggest that drug binding disrupts the hydrogen bond formation and causes localized unwinding of the duplex. The interruption of base pairing would produce single-stranded regions that, under the conditions of low ionic strength in the gels, would collapse and reduce the effective length of the DNA's.

Although comparison of Fig. 1, a and b, would suggest differences in the effects of *cis*- and *trans*-DDP on the electrophoretic mobilities of the circular DNA's, plots of gel migration against bound platinum per nucleotide (r) (Fig. 2) show otherwise. The minima in these plots ($r \sim 0.10$) are nearly coincident for both isomers. One notable difference, however, is the increase in mobility observed for *cis*-DDP at $r < 0.075$. This reproducible difference in gel mobilities at low r values is not yet understood. It is interesting that the characteristic increase in mobility at low r exhibited by the DNA-*cis*-DDP complex resembles that observed when ethidium bromide binds by intercalation to closed circular DNA.

The effects of *cis*- and *trans*-DDP on the electrophoretic mobilities of the closed circular DNA's are also manifest in gels containing ethidium bromide (*5*) (Fig. 1, c and d). The presence of this intercalator in the gel ensures that the closed circular DNA is wound into a net positive superhelix and suppresses the effect on electrophoretic mobility of changes in the superhelix density in regions of unbound platinum. Figure 3 is a plot of migration against r for these dye gels, from which it is evident that both *cis*- and *trans*-DDP produce cooperative changes in the mobility of the DNA's. The r value at which the cooperative effect occurs is identical with that of the mobility minimum observed when either isomer binds to the closed DNA (Fig. 2). The increased mobilities (Fig. 3) again indicate a partially collapsed structure.

The electrophoretic mobility results suggest that the binding of DDP shortens the length of the DNA. This interpretation has been directly confirmed by electron microscopy. As shown in Fig. 4 for the *cis* isomer, the DNA molecules become more compact with increasing r. The opening and subsequent superhelical twisting of the closed circular DNA is clearly evident. Histograms (not shown) of length measurements show the shortening to be as much as 50 percent of the original DNA size. The precise nature of the platinum-DNA interaction that produces this interesting collapse of the double helix is not known. The binding must be covalent, however, since ionic or hydrogen bonding interactions would be reversed under the high chloride concentrations used to quench the reaction. Weakly bound platinum would also separate from DNA in gel electrophoresis.

GARY L. COHEN
WILLIAM R. BAUER
Department of Microbiology, State University of New York, Stony Brook 11794
JACQUELINE K. BARTON
STEPHEN J. LIPPARD
Department of Chemistry, Columbia University, New York 10027

References and Notes

1. B. Rosenberg, L. Van Camp, J. E. Trosko, V. H. Mansour, *Nature (London)* **222**, 385 (1969).
2. For recent reports on the clinical status of the drug, see *Wadley Med. Bull.* **7** (1977).
3. A. J. Thomson, *Platinum Met. Rev.* **21**, 2 (1977).
4. G. Y. H. Chu, S. Mansy, R. E. Duncan, R. S. Tobias, *J. Am. Chem. Soc.* **100**, 593 (1978).
5. M. Howe-Grant, K. C. Wu, W. R. Bauer, S. J. Lippard, *Biochemistry* **15**, 4339 (1976).
6. S. Mickel and W. R. Bauer, *J. Bacteriol.* **127**, 644 (1976).
7. J. Vinograd and J. Lebowitz, *J. Gen. Physiol.* **49**, 103 (1966).
8. J. T. Finch *et al.*, *Nature (London)* **269**, 29 (1977).
9. G. Felsenfeld, *ibid.* **271**, 115 (1978).
10. G. B. Kauffman and D. O. Cowan, *Inorg. Synth.* **7**, 239 (1973).
11. R. W. Davis, M. Simon, N. Davidson, *Methods Enzymol.* **21**, 413 (1971).
12. M. C. Lim and R. B. Martin, *J. Inorg. Nucl. Chem.* **38**, 1911 (1976).
13. L. P. G. Wakelin, *Biochem. Soc. Trans.* **2**, 866 (1974).
14. M. Neal and J. Florini, *Anal. Biochem.* **55**, 328 (1973).
15. Supported by PHS grants CA-15826 from the National Cancer Institute (to S.J.L.) and GM-21176 from the National Institute of General Medical Sciences (to W.R.B.) and by NSF grant PCM76-01839 (to W.R.B.). J.K.B. thanks the National Science Foundation for a predoctoral fellowship. A generous gift of K_2PtCl_4 from Engelhard Industries used to prepare *cis*- and *trans*-DDP is gratefully acknowledged.

21 September 1978; revised 18 December 1978

Regional Localization of the Gene for Human Phosphoribosylpyrophosphate Synthetase on the X Chromosome

Abstract. *Sixty-eight independent hybrid clones were isolated after irradiated normal human lymphocytes were fused with Chinese hamster fibroblasts lacking hypoxanthine-guanine phosphoribosyltransferase activity. The cells were grown under selective conditions requiring retention of the X chromosome–linked locus for human hypoxanthine-guanine phosphoribosyltransferase. The frequency and patterns of cotransference of human phosphoribosylpyrophosphate synthetase with the selected marker and with additional X-linked enzymatic markers confirm X linkage of the structural gene for human phosphoribosylpyrophosphate synthetase and support assignment of this gene to a position on the long arm of the X, between the loci for α-galactosidase and hypoxanthine-guanine phosphoribosyltransferase.*

5 - Phosphoribosyl - 1 - pyrophosphate (PRPP) is an activated sugar phosphate utilized in the synthesis of purine, pyrimidine, and pyridine nucleotides. PRPP is a substrate common to the initial reaction of the pathway of purine synthesis de novo, catalyzed by amidophosphoribosyltransferase (E.C. 2.4.2.14), and to purine base salvage reactions catalyzed by hypoxanthine-guanine phosphoribosyltransferase (HGPRT; E.C. 2.4.2.8) and adenine phosphoribosyltransferase (E.C. 2.4.2.7). That PRPP availability has a role in the regulation of the rate of purine synthesis de novo is suggested by the observations that (i) PRPP is a limiting substrate and an allosteric activator of amidophosphoribosyltransferase (*1*), the presumed rate-limiting enzyme of the pathway, and (ii) increased concentrations of PRPP are found in cells derived from individuals with increased purine nucleotide production caused by either excessive PRPP synthetase (E.C. 2.7.6.1) activity (*2*) or deficiencies (severe or partial) of HGPRT (*3*).

The enzyme PRPP synthetase catalyzes the synthesis of PRPP from adenosine triphosphate (ATP) and ribose-5-phosphate in a complex reaction requiring inorganic phosphate and Mg^{2+}. Human erythrocyte PRPP synthetase consists of a single subunit species capable of reversible self-association to a variety of aggregated forms in a process mediated by effectors of enzyme activity and by enzyme concentration (*4*). Several families have been identified in which structurally altered forms of PRPP synthetase with increased enzyme activity result in increased PRPP production, excessive purine nucleotide and uric acid synthesis, and clinical gout (*2*). Fibroblast cultures from heterozygous female members of two of these families have been shown, by direct and indirect means (*5*), to contain two clones of cells expressing distinct phenotypes with respect to PRPP synthetase. This finding is in accord with the specific chimerism predicted for females heterozygous for X-linked traits by the Lyon hypothesis (*6*) of random X-chromosome inactivation and

0036-8075/79/0309-1016$00.50/0

supports assignment of the structural gene for human PRPP synthetase to the X chromosome. According to the same rationale, loci for other human biochemical markers such as glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49), α-galactosidase (α-GAL; E.C. 3.2.1.22), and HGPRT have previously been assigned to the X chromosome (*7*). Subsequent independent studies of the expression of these human markers in interspecific human-rodent hybrids which retain the human X chromosome have confirmed these assignments (*8*) and have allowed the development of strategies (*9*) to delineate the sequence of genes on this chromosome.

In the studies described here, we used one such strategy in order to confirm X linkage of the gene for human PRPP synthetase and in order to map this locus with respect to the loci for other X-linked markers. Interspecific hybrid clones containing varying lengths of the human X chromosome were derived from fusion of irradiated human lymphocytes and Chinese hamster fibroblasts and were studied for the expression of human PRPP synthetase and other known X-linked human biochemical markers. The purpose of irradiating the human lymphocytes prior to cell fusion was to produce hybrids initially containing a set of broken and otherwise rearranged human chromosomes. Fragments of the X chromosome that are no longer linked to the selected marker, HGPRT, are, like autosomes, rapidly lost from these hybrids (*10*). Different hybrids are therefore found to retain different combinations of human X-linked genes, and it is possible, from a consideration of the map of these genes, to deduce which portion of the X chromosome has been retained in each case. As a result of these studies, X linkage of human PRPP synthetase has been confirmed, and the locus for this enzyme has been assigned to the long arm of the X chromosome between the loci for α-GAL and HGPRT.

The hybrid clones were prepared as described (*9*). Briefly, normal human lymphocytes were purified from the venous blood of a single male individual by the Isopaque-Ficoll technique and were exposed to 0, 1, 2, or 4 kilorads of γ radiation from a double cesium source. Immediately after irradiation, the lymphocytes were fused in the presence of inactivated Sendai virus with Wg3-h Chinese hamster fibroblasts, a DON line derivative lacking HGPRT. Immediately after fusion, cells were distributed into small Falcon flasks containing Eagle's minimum essential medium (MEM). One day later, the medium was changed to MEM containing hypoxanthine ($6 \times 10^{-5}M$), aminopterin ($4.5 \times 10^{-7}M$), thymidine ($1 \times 10^{-5}M$), and glycine ($4 \times 10^{-4}M$) (HAT medium). The unfused lymphocytes were washed away during the medium change, and any hamster cells that had not received a human X-chromosome component carrying the gene for HGPRT were killed in the selective HAT medium. Human-hamster hybrids that retained the human HGPRT gene grew up as clones, one of which was isolated from each Falcon flask. It is thus certain that each hybrid clone used in the following analysis was the product of a separate fusion event. The clones were assayed for the presence of various human enzymes, and, on the basis of the initial results for known X-linked enzymes, a subset of clones was chosen as likely to be informative in the regional localization of the gene for PRPP synthetase. These clones were maintained in HAT medium, except where specifically indicated. They were tested for the presence of human PRPP synthetase and for each of the following X-linked human enzymes: phosphoglycerokinase (PGK; E.C. 2.7.2.3), α-GAL, HGPRT, and G6PD. With the exception of human PRPP synthetase, the human forms of these enzymes can readily be separated from their hamster counterparts by electrophoresis. Human PGK, HGPRT, and G6PD were identified by Cellogel electrophoresis as described by Meera Khan (*11*). Human α-GAL was identified by the hydrolysis of the fluorigenic substrate 4-methyl-umbelliferyl-α-D-galactopyranoside by agarose gel electrophoresis according to the method of Bakay *et al.* (*12*). Despite attempts to separate human and hamster PRPP synthetases by electrophoresis in several buffer systems of differing *p*H, no reliable difference was detected. Therefore, human PRPP synthetase was detected by specific immunochemical inactivation of the enzyme with the concentrated immunoglobulin G (IgG) fraction of serum from rabbits immunized with purified normal human erythrocyte PRPP synthetase (*13*).

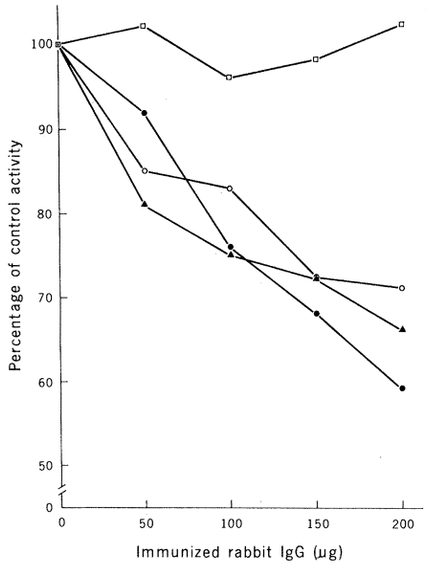

Fig. 1. Neutralization of PRPP synthetase activity by immunized rabbit IgG. Cell extracts were prepared by freezing and thawing cells suspended in 8 m*M* sodium phosphate, 10 m*M* glutathione, and 1 m*M* EDTA (*p*H 7.4) three times in liquid nitrogen. After centrifugation at 27,000*g* for 40 minutes, the supernatant layers containing 0.1 to 0.2 mg of protein were added to an incubation mixture at *p*H 7.4 which contained the following in a volume of 100 μl: sodium phosphate buffer (*p*H 7.4), 2.2 μmole; NaCl, 15.4 μmole; bovine serum albumin, 0.5 mg; reduced glutathione, 0.3 μmole; EDTA, 0.03 μmole; and the appropriate volume of either unimmunized or immunized rabbit IgG (5 mg/ml). Incubation and subsequent assay for PRPP synthetase activity were carried out as previously described (*14*). Sources of cell extracts were: Chinese hamster fibroblast line Wg3-h (□); human lymphoblast line WI-L2 (●); human-hamster hybrid cell lines 2D (○) and R4-65 (▲). Both R4-65 and 2D also expressed the human enzymes HGPRT, G6PD, and α-GAL. In addition, 2D expressed human PGK and contained a human X chromosome identifiable by trypsin-Giemsa banding (*18*). The comparable decrements in PRPP synthetase activities in the human lymphoblast extract and the extracts from the hybrid cell lines could reflect either neutralization of activity in hamster-human enzyme complexes in the hybrid cell extracts or suppression of hamster PRPP synthetase activity in these hybrids.

The rabbit antiserum to PRPP synthetase was prepared and characterized as described in (*14*) but with these modifications: rabbits received 200 μg of purified PRPP synthetase in complete Freund's adjuvant injected subcutaneously into the footpads on two occasions separated by 1 week; subsequently they received 100 μg of purified enzyme in incomplete Freund's adjuvant injected intradermally into several sites in the shoulder regions. Serums of the immunized rabbits, and IgG fractions of these serums, were shown to contain specific antibody to PRPP synthetase by gel diffusion and immunotitration analyses (*14*). Immunochemical inactivation of PRPP synthetase activity was studied as previously described (*14*) in human erythrocyte, fibroblast, and lymphoblast extracts, and in extracts from the parental hamster fibroblast line Wg3-h, as well as from each of the interspecific hybrid clones. For each point in these titration studies (Fig. 1), a control incubation was carried out in which the IgG fraction from the serum of unimmunized rabbits replaced the specific immune IgG. Results of the inactivation studies were expressed as

Table 1. The retention of human PRPP synthetase in human-hamster hybrid clones containing different fragments of the human X chromosome. (The parental human cells were subjected to various doses of γ radiation before cell fusion.)

Human enzymes present				Number of clones examined	Clones expressing human PRPP synthetase	Clones lacking human PRPP synthetase
PGK	α-GAL	HGPRT	G6PD			
+	+	+	+	22	22	0
+	+	+	−	11	11	0
−	+	+	−	6	5	1
−	−	+	−	4	2	2
−	−	+	+	17	5	12
−	+	+	+	8	8	0

the percentage of control activity remaining after incubation.

As shown in Fig. 1, immunized rabbit IgG did not significantly inactivate PRPP synthetase in extracts from the parental hamster line Wg3-h but did inactivate PRPP synthetase in extracts of human lymphoblast line WI-L2. Enzyme activity remaining after titration of the human lymphoblast extract was demonstrated to represent residual activity in the enzyme-antibody complex by means of complete removal of this activity after precipitation of rabbit antiserum with specific goat antiserum to rabbit IgG. In contrast, addition of goat antiserum to rabbit IgG did not remove PRPP synthetase activity from extracts of the hamster fibroblast line Wg3-h containing equivalent amounts of rabbit antiserum. The presence of human PRPP synthetase in interspecific hybrid clones, as assessed by specific immunochemical inactivation, is shown in Fig. 1 for two representative hybrid clones. Hybrid clones showing less than 15 percent inactivation of total PRPP synthetase in the presence of immunized rabbit IgG were recorded as lacking human PRPP synthetase activity.

It has been shown (*10*) that hybrids between Wg3-h and irradiated human lymphocytes tend rapidly to lose any human genes that are not syntenic with the selected, X-linked locus, HGPRT. In the present study, out of 27 hybrid clones made with lymphocytes exposed to 1 krad, 26 were found to have retained human PRPP synthetase, thereby providing a strong indication that this enzyme is X linked. The following back-selection experiment was used to confirm the synteny of PRPP synthetase with known X-linked enzymes. Three hybrid clones which had retained human PRPP synthetase and the human X-linked enzymes PGK, α-GAL, HGPRT, and G6PD were transferred to MEM containing hypoxanthine, thymidine, and glycine, as in HAT medium, but lacking aminopterin. After 2 days (approximately three generations) in this nonselective medium, the cells were resuspended and returned to growth in either HAT medium or MEM containing 30 μM thioguanine, the latter being a medium that selects against cells containing HGPRT activity. Subclones were isolated in each medium and were tested for the retention of their human enzymes. While all eight subclones isolated from HAT medium retained each of the human enzymes originally detected in the parental clones, 11 out of 11 independent subclones isolated in thioguanine were found to have lost PRPP synthetase and all four X-linked enzymes. In previous studies (*10*), loss of autosomal markers did not frequently accompany loss of HGPRT and other X-linked markers during back-selection in medium containing thioguanine. The simultaneous loss of five X-linked human enzymes occurred with a high frequency (about 1 in 10^3 hybrid cells) in the current studies, suggesting that the thioguanine-resistant subclones arose by chromosome loss, rather than by mutation at the HGPRT locus. In this case, the uniform cosegregation of human PRPP synthetase with four human X-linked genes is additional evidence that PRPP synthetase is itself X linked.

Table 1 shows how the retention of the gene for PRPP synthetase varies among hybrids that retain different portions of the X chromosome extending in either direction from the HGPRT locus. The genes used as markers for these different lengths of chromosome are believed to be situated on the long arm of the X in the order PGK–α-GAL–HGPRT–G6PD, with PGK nearest to the centromere (*9*). Table 1 also shows that PRPP synthetase is frequently retained in hybrids that have lost either the proximal part of the X chromosome bearing the PGK and α-GAL loci or the distal tip of the long arm, bearing the G6PD locus. It follows that the gene for PRPP synthetase must lie between α-GAL and G6PD. It is extremely unlikely that the PRPP synthetase gene lies between HGPRT and G6PD, since there are 12 clones that have lost PRPP synthetase and yet have retained G6PD. Taken together, these results indicate that the PRPP synthetase locus must lie between the loci for HGPRT and α-GAL. Whenever the X chromosome is broken between the loci for PRPP synthetase and HGPRT, the resulting loss of PRPP synthetase should be accompanied by the loss of both α-GAL and PGK. This prediction is borne out, in that all 15 clones lacking PRPP synthetase were found to have lost PGK, and 14 of the 15 had also lost α-GAL. The single clone lacking PRPP synthetase but retaining α-GAL is the only clone in the 68 analyzed that is apparently at variance with the assignment of the PRPP synthetase locus between α-GAL and the selected locus. However, the pattern of markers in this clone, which received 2 krad of radiation, may have been the result of radiation-induced interstitial deletion, the occurrence of which has been suggested in previous studies with this mapping technique (*10*). In fact, all four subclones selected from this clone in MEM containing 30 μM thioguanine lost both human α-GAL and HGPRT, whereas three other subclones selected in parallel in HAT medium retained both markers, suggesting that in this clone α-GAL and HGPRT loci are still located on the same segment of the X chromosome.

The following observations are also relevant to the regional localization of the PRPP synthetase gene. A sixth X-linked human gene, that specifying the cell surface antigen, SA-X, has previously been assigned to the region between the HGPRT and G6PD loci, by use of an analysis similar to that presented in the preceding paragraph (*15*). Thirteen of the clones used to make that assignment were included in the present studies. Of these, three were found to have retained human PRPP synthetase despite having lost SA-X, while one had lost PRPP synthetase but retained SA-X. The simplest explanation for the independent segregation of these two loci is that they are situated on opposite sides of the selected marker, HGPRT. Further information was obtained from a hybrid clone selected in HAT medium from a fusion between Wg3-h cells and GM-97, a human fibroblast strain bearing a reciprocal translocation between chromosome 1 and the X chromosome. This translocation separates the tip of the long arm of the X, bearing the HGPRT and G6PD loci, from the proximal portion, bearing the α-GAL and PGK genes (*16*). The hybrid was found to express human HGPRT and G6PD, but it lacked human PGK, α-GAL, and PRPP synthetase. This constitutes preliminary evidence

excluding the PRPP synthetase gene from the region of the X chromosome distal to the point of translocation, namely Xq26.

Assignment of the structural locus for PRPP synthetase to a position on the X chromosome segment between the centromere and the locus for HGPRT, closer to the locus for HGPRT than other known markers in this segment, raises interesting possibilities concerning a functional role for this arrangement of loci coding for sequential enzymes of PRPP metabolism. On the basis of studies on the PRPP synthetase levels in fibroblasts deficient in HGPRT, it has previously been proposed that the HGPRT locus may have some regulatory effect on the production of PRPP synthetase (*17*). Unfortunately, since the clones used in the present study were not a random set, but were specially chosen, it is not possible to apply the method of Goss and Harris (*10*) to estimate the distance between the PRPP synthetase and HGPRT genes with any reliability. Only further studies will make it possible to judge whether the apparent proximity of these two genes could be important in the regulation of PRPP synthesis and utilization.

MICHAEL A. BECKER
RICHARD C. K. YEN
PAMELA ITKIN
Rheumatology Division, Department of Medicine, Veterans Administration Hospital, San Diego, California 92161 and *University of California, San Diego, La Jolla 92093*
STEPHEN J. GOSS
Sir William Dunn School of Pathology, University of Oxford, Oxford, OX 1 3RE, United Kingdom
J. EDWIN SEEGMILLER
Department of Medicine, University of California, San Diego
BOHDAN BAKAY
Department of Pediatrics, University of California, San Diego

References and Notes

1. E. W. Holmes, J. A. McDonald, J. M. McCord, J. B. Wyngaarden, W. N. Kelley, *J. Biol. Chem.* **248**, 144 (1973).
2. O. Sperling, G. Eilam, S. Persky-Brosh, A. deVries, *Biochem. Med.* **6**, 310 (1972); M. A. Becker, L. J. Meyer, A. W. Wood, J. E. Seegmiller, *Science* **179**, 1123 (1973); M. A. Becker, *J. Clin. Invest.* **57**, 308 (1976); E. Zoref, A. deVries, O. Sperling, *ibid.* **56**, 1093 (1975).
3. F. M. Rosenbloom, J. F. Henderson, I. C. Caldwell, W. N. Kelley, J. E. Seegmiller, *J. Biol. Chem.* **243**, 1166 (1968); M. L. Greene and J. E. Seegmiller, *J. Clin. Invest*, **48**, 32a (1969); A. W. Wood, M. A. Becker, J. E. Seegmiller, *Biochem. Genet.* **9**, 261 (1973).
4. M. A. Becker, L. J. Meyer, W. H. Huisman, C. S. Lazar, W. B. Adams, *J. Biol. Chem.* **252**, 3911 (1977).
5. R. C. K. Yen, W. B. Adams, C. Lazar, M. A. Becker, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 482 (1978); E. Zoref, A. deVries, O. Sperling, *Hum. Hered.* **27**, 73 (1977).
6. M. F. Lyon, *Nature (London)* **190**, 372 (1961).
7. R. G. Davidson, H. M. Nitowsky, B. Childs, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 481 (1963); G. Romeo and B. R. Migeon, *Science* **170**, 180 (1970); F. M. Rosenbloom, W. N. Kelley, J. F. Henderson, J. E. Seegmiller, *Lancet* **1967-II**, 305 (1967).
8. M. Nabholz, V. Miggiano, W. Bodmer, *Nature (London)* **223**, 358 (1969); F. H. Ruddle, V. M. Chapman, F. Ricciuti, M. Murnane, R. Klebe, P. Meera Khan, *Nature (London) New Biol.* **232**, 69 (1971); K. H. Grzeschik, A. M. Grzeschik, S. Banhof, G. Romeo, M. Siniscalco, H. van Someren, P. Meera Khan, A. Westerveld, R. Bootsma, *ibid.* **240**, 48 (1972).
9. S. J. Goss and H. Harris, *Nature (London)* **255**, 680 (1975); P. S. Gerald and J. A. Brown, *Cytogenet. Cell Genet.* **13**, 29 (1974).
10. S. J. Goss and H. Harris, *J. Cell. Sci.* **25**, 17 (1977).
11. P. Meera Khan, *Arch. Biochem. Biophys.* **145**, 470 (1971).
12. B. Bakay, M. Graf, S. Carey, E. Nissinen, W. L. Nyhan, *Biochem. Genet.* **16**, 227 (1978).
13. I. H. Fox and W. N Kelley, *J. Biol. Chem.* **246**, 5739 (1971).
14. M. A. Becker, P. J. Kostel, L. J. Meyer, J. E. Seegmiller, *Proc. Natl. Acad. Sci U.S.A.* **70**, 2749 (1973); M. A. Becker, P. J. Kostel, L. J. Meyer, *J. Biol. Chem.* **250**, 6822 (1975).
15. D. W. Buck, S. J. Goss, W. F. Bodmer, *Cytogenet. Cell Genet.* **16**, 99 (1976). The X-linked human surface antigen SA-X has been mapped between the loci for HGPRT and G6PD by the method described here. However, an accurate determination of the map distance between these two markers has not been made.
16. H. H. Punnett, M. L. Kistermacher, A. E. Greene, L. L. Coriell, *Cytogenet. Cell Genet.* **13**, 406 (1974); E. Seravalli, *et al.*, *ibid.* **16**, 219 (1976). The GM-97 cells were obtained from the Genetic Mutant Cell Repository, Camden, N.J.
17. D. W. Martin and B. A. Maler, *Science* **193**, 408 (1976).
18. L. L. Deaven and D. F. Peterson, *Chromosoma* **41**, 129 (1973).
19. This work was supported in part by the Medical Research Service of the Veterans Administration, by a grant (to J.E.S.) from the Kroc Foundation, and by grant AM-18197 (to M.A.B.) and grant GM-17702 (to J.E.S.) from the National Institutes of Health and grant BMS 74-21424 from NSF (to B.B.). We thank C. S. Lazar, J. Kerr-Harnott, and W. B. Adams for technical assistance and A. Goodman for preparation of the manuscript.

12 July 1978; revised 6 October 1978

Methylation of Mouse Liver DNA Studied by Means of the Restriction Enzymes Msp I and Hpa II

Abstract. *The restriction enzymes Hpa II and Msp I both recognize the sequence 5'-CCGG (C, cytosine; G, guanine). However, Hpa II cuts mouse liver DNA to fragments four times larger than does Msp I. The size of DNA cut by Msp I is close to that predicted from base composition and nearest neighbor analysis. The most probable explanation of these results is that in mouse the site 5'-CCGG is highly methylated.*

5-Methylcytosine (5-MeCyt) is the only minor base found in vertebrate DNA (3 to 10 percent of cytosine), and is known to result from the enzymatic methylation of cytosine at the DNA level (*1*). The function of this modified base is not known although there has been speculation that it may be involved in differentiation (*2*).

Nearest neighbor and pyrimidine tract analysis have shown that 5-MeCyt occurs most often in the dinucleotide CpG (C, cytosine; G, guanine) (*3*), but little more is known about the sequence specificity of DNA methylation. Several groups have used restriction enzymes to probe the distribution of 5-MeCyt in eukaryotic DNA, the rationale being that 5-MeCyt in the site recognized by a DNA restriction enzyme would prevent cutting of DNA at that site (*4-6*). Gautier *et al.* (*5*) found that the restriction enzymes Hha I, Hpa II, and Sma I cut calf thymus satellite DNA only after the DNA had been cloned, thereby altering its pattern of methylation. Bird and Southern (*4*) similarly showed that the enzymes Hha I, Hpa II, and Ava I do not cut highly methylated (14 percent 5-MeCyt of the total cytosine) somatic ribosomal DNA of *Xenopus*, but do cut

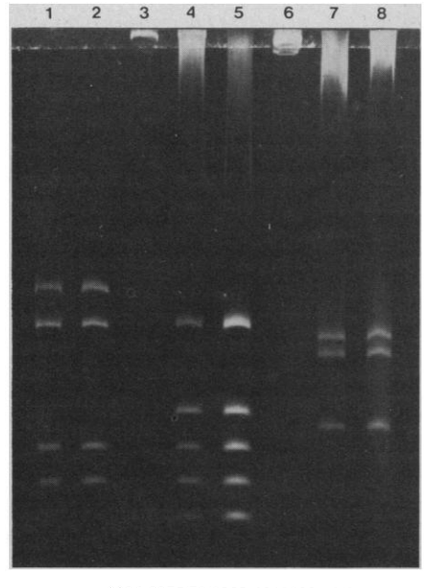

Fig. 1. Comparison of Hpa II and Msp I. One microgram each of ϕX174 pBR333 and pBR345 was treated with 2 units of Hpa II or Msp I (*8*). Mixtures were then subjected to electrophoresis on a 5 percent acrylamide gel, stained with ethidium bromide, and photographed (*10*). (Channel 1) pBR345 + Msp I; (Channel 2) pBR345 + Hpa II; (Channel 3) pBR345 uncut; (Channel 4) pBR333 + Msp I; (Channel 5) pBR333 + Hpa II; (Channel 6) pBR333 uncut; (Channel 7) ϕX174 plus Msp I; (Channel 8) ϕX174 + Hpa II.

0036-8075/79/0309-1019$00.50/0