identification of AFL as the major plasma metabolite of AFB_1 in the rat indicates that AFL-forming activity in vitro may be useful as a metabolic parameter for the estimation of animal and human susceptibilities to the carcinogenic effects of AFB_1. Similar studies with other species with different susceptibilities are warranted.

Z. A. WONG
D. P. H. HSIEH

Department of Environmental Toxicology, College of Agricultural and Environmental Sciences, University of California, Davis 95616

References and Notes

1. P. M. Newberne, *Environ. Health Perspect.* **9**, 1 (1974).
2. G. N. Wogan, *Methods Cancer Res.* **7**, 309 (1973).
3. T. C. Campbell and L. Stoloff, *J. Agric. Food Chem.* **22**, 1006 (1974).
4. R. C. Garner, *FEBS Lett.* **36**, 261 (1973); ———, E. C. Miller, J. A. Miller, *Cancer Res.* **32**, 2058 (1972); D. H. Swenson, J. K. Lin, E. C. Miller, J. A. Miller, *ibid.* **37**, 172 (1977).
5. This was previously called the AFB_1 2,3-epoxide but has been renumbered in accordance with recommendations of the International Union of Pure and Applied Chemistry.
6. C. N. Martin and R. C. Garner, *Nature (London)* **267**, 863 (1977); J. M. Essigman, R. G. Croy, A. M. Nadzan, W. F. Busby, V. N. Reinhold, G. Buchi, G. N. Wogan, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1870 (1977); D. H. Swenson, J. A. Miller, E. C. Miller, *Cancer Res.* **35**, 3811 (1975).
7. R. S. Portman, K. M. Plowman, T. C. Campbell, *Biochim. Biophys. Acta* **208**, 487 (1970).
8. D. S. P. Patterson, *Food Cosmet. Toxicol.* **11**, 287 (1973).
9. T. C. Campbell and J. R. Hayes, *Toxicol. Appl. Pharmacol.* **35**, 199 (1976).
10. A. S. Salhab and G. S. Edwards, *J. Toxicol. Environ. Health* **2**, 583 (1977).
11. P. M. Loveland, R. O. Sinnhuber, K. E. Berggren, L. M. Libbey, J. E. Nixon, N. E. Pawlowski, *Res. Commun. Chem. Pathol. Pharmacol.* **16**, 167 (1977).
12. A. S. Salhab and D. P. H. Hsieh, *ibid.* **10**, 419 (1975).
13. J. J. Wong and D. P. H. Hsieh, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2241 (1976).
14. D. P. H. Hsieh, Z. A. Wong, J. J. Wong, C. Michas, B. H. Ruebner, in *Mycotoxins in Human and Animal Health*, J. V. Rodricks, C. W. Hesseltine, M. A. Mehlman, Eds. (Pathodox Publishers, Park Forest South, Ill., 1977), p. 37.
15. I. F. H. Purchase, *Food Cosmet. Toxicol.* **10**, 531 (1972).
16. T. C. Campbell and L. Salamat, in *Mycotoxins in Human Health*, I. F. H. Purchase, Ed. (Macmillan, London, 1971), p. 270.
17. R. C. Garner, E. C. Miller, J. A. Miller, *Cancer Res.* **32**, 2058 (1972); N. E. Pawlowski, G. L. Schoenhard, D. J. Lee, L. M. Libbey, P. M. Loveland, R. O. Sinnhuber, *J. Agric. Food Chem.* **25**, 437 (1977).
18. D. S. P. Patterson and B. A. Roberts, *Food Cosmet. Toxicol.* **10**, 501 (1972).
19. A. S. Salhab and G. S. Edwards, *Cancer Res.* **37**, 1016 (1977).
20. Z. A. Wong and D. P. H. Hsieh, paper presented at the first meeting of the International Congress on Toxicology, Toronto, Canada (1977).
21. ———, in preparation.
22. W. F. Cuthbertson, A. C. Laursen, D. A. H. Pratt, *Br. J. Nutr.* **21**, 893 (1967); R. H. Adamson, P. Correa, S. M. Seiber, K. R. McIntire, D. W. Dalgard, *J. Natl. Cancer Inst.* **50**, 549 (1976).
23. G. S. Edwards, T. D. Rintel, C. M. Parker, *Proc. Am. Assoc. Cancer Res.* **16**, 133 (1975).
24. G. L. Schoenhard, thesis, Oregon State University (1974).
25. D. P. H. Hsieh and R. I. Mateles, *Appl. Microbiol.* **22**, 79 (1971).
26. We thank J. J. Wong, D. W. Rice, and M. Y. Fukayama for their experimental assistance. Supported by National Institute of Environmental Health Sciences training grant ES 00125 and Western Regional Research Project W-122.

26 September 1977; revised 12 December 1977

Hv(1), a Variable-Region Genetic Marker of Human Immunoglobulin Heavy Chains

Abstract. *A new antigenic determinant was discovered with a hemagglutination-inhibition assay system. Designated Hv(1), it is located in the variable region of human immunoglobulin heavy chains of the G, M, and A classes. Pedigree and population analyses suggest that it has an autosomal dominant mode of inheritance. This represents the first description of an allotypic determinant in the variable region of human immunoglobulins.*

The inherited polymorphisms present in antigenic determinants on immunoglobulin molecules have aided the study of the genetic control of antibody synthesis. Two of the more successful approaches for examining the diversity and heritability of immunoglobulin genes are primary structure and serologic analyses. The former approach has categorized variable-region genes by their location on heavy chains (V_H) and light chains (V_κ and V_λ); delineated specific subgroups of these three gene families; and localized hypervariable regions, which account for the specificity of the antigen-combining site (*1*). Primary structure analysis is limited by its need for large amounts of homogeneous immunoglobulin. The serologic approach, which does not require isolated immunoglobulin, has been useful in defining constant-region allotypes (that is, inherited antigenic determinants of proteins that are present in some individuals but not in others) in both animal and human immunoglobulin (*2*); variable-region subgroups of human light (*3*) and heavy (*4*) chains; and variable-region allotypes in rabbit immunoglobulins (*5*). Allotypes in the variable regions of human immunoglobulin have not been defined by either of these methods. We now describe the use of an indirect hemagglutination inhibition system to define a human immunoglobulin variable-region genetic marker, and to characterize its mode of inheritance.

Rabbit antiserum to the immunoglobulin M (IgM) protein McE was rendered specific by solid phase immunoadsorption on a column of Sepharose 4B coupled with five different monoclonal IgM proteins (*6*). The serum was further adsorbed with washed, type O, Rh+ erythrocytes. Complement in the serum was inactivated by heating at 56°C for 30 minutes. Monoclonal immunoglobulins of the G, A, and D classes (IgG, IgA, and IgD) were isolated from patients with multiple myeloma, and IgM was isolated from patients with Waldenstrom's macroglobulinemia (*7*). Heavy and light chains were prepared by reducing purified immunoglobulins with 0.01*M* dithiothreitol in 0.1*M* tris-Hcl at 23°C for 2 hours; the component chains were separated on Sephadex G-100 (*8*). The Fab and Fc fragments of IgG (Fabγ and Fcγ) were prepared and isolated (*9*). The Fab and Fc fragments of IgM [Fabμ and (Fc)5μ] were prepared (*10*), separated on Sephadex G-200, and adsorbed on Sepharose 4B coupled with monospecific antibodies to human κ chains.

Hemagglutination inhibition (*11*) was performed with type O, Rh+ erythrocytes, coupled by chromium chloride with intact immunoglobulins or their Fab fragments. Optimal coating was achieved with 1 to 5 mg protein per milliliter and 0.025 to 0.1 percent chromium chloride. In a typical coating experiment, 0.05 ml intact immunoglobulin or Fab solution was added to an equal volume of packed erythrocytes. The mixture was shaken for 1 minute, 0.05 ml of chromium chloride solution was added, and the mixture was shaken for an additional 5 minutes. Coated cells were washed at least three times with a 30-volume excess of 0.15*M* NaCl, and they were suspended to 0.2 percent in 0.15*M* NaCl containing 0.1 percent gelatin.

The absorbed antiserum had an agglutinating titer ranging from 1:128 to 1:512 for red blood cells coated with McE IgM(κ) or its Fabμ fragment, as well as for cells coated with an IgG1(κ) myeloma protein (FER); however, it did not agglutinate cells coated with six other monoclonal immunoglobulins. In order to determine the distribution of antigenic determinants recognized by the antiserum, we tested a panel of 97 isolated human monoclonal immunoglobulins. Approximately 25 percent of the IgG, 16 percent of the IgA, and 29 percent of the IgM inhibited agglutination (Table 1), indicating that the antigenic determinant was not restricted to any particular immunoglobulin class. This was consistent with our previous finding that the same variable-region amino acid sequence was able to associate with constant regions of different heavy-chain classes (*12*). The number of IgD proteins examined was not large enough to give any significant information. Sequence studies from the amino-terminal ends of four inhibiting heavy chains having glutamic acid as

0036-8075/78/0421-0327$00.50/0

Table 1. Purified monoclonal human immunoglobulins inhibit hemagglutination of erythrocytes coated with McE Fab by the absorbed antiserum directed toward McE IgM(κ).

Protein	Samples (No.)		Frequency (%)
	Tested	Inhibiting	
IgG	60	15	25
IgA	18	3	16
IgM	17	5	29
IgD	2	0	0
κ*	59	15	25
λ*	38	8	21
H(B)†	32	11	34
H(U)†	29	4	13

*Immunoglobulins with κ and λ types of L chains, respectively. †Immunoglobulins with blocked (V_HI, II, and IV) and unblocked (V_HIII) H chains respectively.

the first residue (unblocked) showed that one of them (Eva μ) belonged to the V_H subgroup V_HIII (*13*), and that a second one (Arp α) probably belonged to the recently described unblocked V_HI sub-subgroup (*14*); the remaining two had more sequence homology with the prototype of V_HIII than with the unblocked V_HI sub-subgroup. Our data further indicated that the antigenic determinants detected by this hemagglutination-inhibition system were not restricted to one variable-region subgroup, but that they might be preferentially associated with immunoglobulins having blocked (pyrrolidone carboxylic acid as the first residue) heavy chains. The determinant did not appear to be associated with light chain types since hemagglutination was inhibited by approximately 25 percent of immunoglobulins with κ chains and 21 percent of those with λ chains.

To determine the location of this antigenic determinant in the immunoglobulin molecule, Fab and Fc fragments as well as heavy and light chains of proteins McE and FER were employed as inhibitors (Table 2). The Fab fragments and heavy chains of both proteins inhibited hemagglutination, but the corresponding Fc fragments and light chains did not. These data, in combination with those presented in Table 1, suggested that the antigenic determinant was located within the variable region of the heavy chain.

In a population study, serial dilutions of normal human serums were tested for inhibitory activity. Of 155 serums from unrelated normal persons, 40 inhibited beyond the 1:512 dilution, 71 failed to inhibit at the 1:4 dilution, and the remainder inhibited at intermediate values (*15*). Since a serum dilution of 1:16 had been routinely used as a criterion for immunoglobulin constant-region allotypes, we arbitrarily assigned those serums inhibiting beyond a 1:16 dilution as positive for the antigenic determinant. Based on this, 53 out of 155 unrelated individuals were positive, approximating the frequency of the antigenic determinant in the general population to be 34 percent. If this antigenic determinant is inherited as a simple dominant Mendelian trait, the frequency of the gene coding for the determinant, designated Hv(1)+, is estimated to be 0.189 ± 0.023, and that of its alternative allele, Hv(1)−, 0.811 ± 0.023 (*16*). Two possible explanations exist for the quantitative variation of the Hv(1) determinant seen in normal human serums. The quantity of immunoglobulins carrying the antigenic determinant rather than production of the antigenic determinant itself may be under genetic control. Such examples can be seen in Ir genes (*17*), where responder and nonresponder strains of mice are distinguished partly by the extent of the immune response rather than by its absolute presence or absence. Alternatively, serums that inhibit only at higher concentrations may contain cross-reactive immunoglobulins. Since immunoglobulin variable regions are extremely heterogeneous, the existence of cross-reactive antigenic determinants would be likely.

Table 2. Inhibition of two hemagglutination systems by purified monoclonal immunoglobulins, their fragments, and polypeptide chains.

Protein or fragment used as inhibitor	Cells coated with	
	FER IgGl(κ)	MCE IgM(κ)
FER IgGl(κ)	0.004*	0.015
FER Fab	0.008	0.065
FER Fc	†	†
FER H chain	0.065	0.125
FER L chain	†	†
McE IgM(κ)	0.008	0.002
McE Fab	0.015	0.008
McE Fc	†	†
McE H chain	0.065	0.030
McE L chain	†	†

*Lowest concentration (milligrams per milliliter) giving inhibition. †No inhibition at starting dilution of 0.5 mg/ml.

Genetic transmission of Hv(1) was studied in 12 families, and the pedigrees of four are shown in Fig. 1. Two Hv(1)+ × Hv(1)+, six Hv(1)+ × Hv(1)−, and seven Hv(1)− × Hv(1)− Caucasian matings with tested offspring have been analyzed. Assuming the gene frequency estimate of 0.811 for the Hv(1)− allele and that the population is panmictic with respect to the Hv(1) locus, the expected proportion (Snyder's ratio) of Hv(1)− offspring from Hv(1)+ × Hv(1)+ matings is 0.2006; that of the Hv(1)+ × Hv(1)− mating is 0.4478. Among five offspring from Hv(1)+ × Hv(1)+ matings, one Hv(1)− individual was observed (1.002 expected). Among 22 offspring from Hv(1)+ × Hv(1)− matings, 12 Hv(1)− individuals were observed (9.852 expected). Among 21 offspring from Hv(1)− × Hv(1)− matings, all showed the expected Hv(1)− phenotype except the one female marked by an arrow in Fig. 1. However, the inhibition titer of her serum was 1:64 whereas the majority of the other Hv(1)+ serums had much higher titers. This might be the result of experimental variation, illegitimacy, or a new mutation. Overall, our data support the hypothesis that Hv(1) is determined by a simple dominant Mendelian gene. However, due to the quantitative variation observed in hemagglutination inhibition by normal human serums (*15*), the involvement of multiple loci or regulatory genes cannot be eliminated. Detailed population studies and linkage

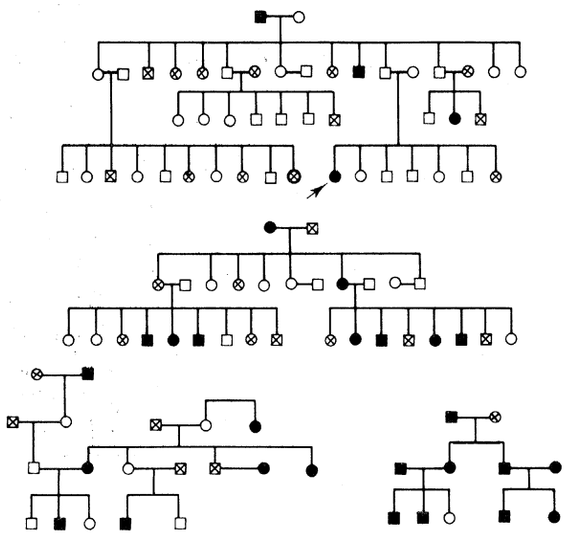

Figure 1. Pedigrees of four families. Closed circles and squares indicate individuals positive for the Hv(1) antigenic determinant. Open circles and squares indicate negative individuals, and those with a cross indicate not available for testing.

studies with known C_H allotypes in families of various races are in progress.

The discovery of the Hv(1) allotypic determinant will be of considerable importance in (i) examining inheritance of immunoglobulin variable-region genes; (ii) evaluating current theories on the origins of antibody diversity (*18*); (iii) exploring the relationship and possible linkage between immunoglobulin variable-region genes and both constant-region and regulatory genes; (iv) elucidating possible associations between variable-region allotypes and certain immunodeficiency, autoimmune, and neoplastic diseases; and (v) examining the relationship between variable-region allotypes and the antigen receptor of T cells (*19*).

A. C. WANG
S. MATHUR
J. PANDEY

Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston 29403

F. P. SIEGAL
C. R. MIDDAUGH
G. W. LITMAN

Sloan-Kettering Institute for Cancer Research, New York 10021

References and Notes

1. J. D. Capra and J. M. Kehoe, *Adv. Immunol.* **20**, 1 (1975); T. T. Wu and E. A. Kabat, *J. Exp. Med.* **132**, 211 (1970).
2. A. G. Steinberg, *Annu. Rev. Genet.* **3**, 25 (1969); R. Grubb, *The Genetic Marker of Human Immunoglobulins* (Springer-Verlag, New York, 1970), pp. 1–152; A. Nisonoff, J. E. Hopper, S. B. Spring, *The Antibody Molecule* (Academic Press, New York, 1975), p. 346; H. G. Kunkel and T. J. Kindt, in *Immunogenetics and Immunodeficiency*, B. Benacerraf, Ed. (University Park Press, Baltimore, 1975), p. 55; H. H. Fudenberg, J. R. L. Pink, D. Stites, A. C. Wang, *Basic Immunogenetics* (Oxford Univ. Press, New York, 1972), p. 48.
3. A. Solomon and C. L. McLaughlin, *J. Exp. Med.* **130**, 1295 (1969).
4. O. Forre, J. B. Natvig, H. G. Kunkel, *ibid.* **144**, 897 (1976); L. Rivat, C. Rivat, J. P. Lebreton, C. Ropartz, *Eur. J. Immunol.* **6**, 624 (1976).
5. R. Mage, R. Lieberman, M. Potter, W. D. Terry, in *The Antigens*, M. Sela, Ed. (Academic Press, New York, 1973), vol. 1, p. 299; T. J. Kindt, *Adv. Immunol.* **21**, 35 (1975).
6. D. Frommel, J. M. Dupuy, G. W. Litman, R. A. Good, *J. Immunol.* **105**, 1292 (1970).
7. A. C. Wang, J. Gergely, H. H. Fudenberg, *Biochemistry* **12**, 512 (1973); C. R. Middaugh, G. J. Thomas, M. E. Prescott, M. E. Aberlin, G. W. Litman, *ibid.* **16**, 2986 (1977).
8. E. Rajnavolgyi, A. C. Wang, G. A. Medgyesi, J. Gergely, *Immunochemistry* **12**, 663 (1975).
9. A. C. Wang and I. Y. Wang, *ibid.* **14**, 197 (1977).
10. A. G. Plaut, N. Calcanico, T. B. Tomasi, *J. Immunol.* **108**, 289 (1972).
11. G. N. Vyas, H. H. Fudenberg, H. F. Pretty, E. R. Gold, *ibid.* **100**, 244 (1968); E. van Loghem, A. C. Wang, J. Shuster, *Vox Sang.* **24**, 481 (1973).
12. A. C. Wang, I. Y. Wang, H. H. Fudenberg, *J. Biol. Chem.* **252**, 7192 (1977).
13. A. C. Wang, H. H. Fudenberg, J. R. L. Pink, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1143 (1971).
14. J. E. Hopper and E. Brahn, *J. Immunol.* **119**, 847 (1977).
15. Nineteen serums inhibited at 1:4, twelve at 1:8, three at 1:16, two at 1:32, two at 1:64, one at 1:128, and five at 1:256 dilutions.
16. C. C. Li, *First Course in Population Genetics* (Boxwood, Pacific Grove, Calif., 1976), p. 20.
17. H. O. McDevitt, K. B. Necjtol, F. C. Grumet, G. F. Mitchell, T. G. Wegmann, *Prog. Immunol.* **1**, 495 (1971).
18. A. J. Cunningham, Ed., *The Generation of Antibody Diversity: A New Look* (Academic Press, New York, 1976), pp. 1–211.
19. H. Binz, H. Wigzell, H. Bazin, *Nature (London)* **264**, 639 (1976).
20. We thank E. Tung and K. Shah for technical assistance, Dr. S. H. Schuman from the Family Practice Division of the Medical University of South Carolina (MUSC) and Dr. S. H. Chen from the Department of Pediatrics of the University of Washington, Seattle, for serums from pedigreed families. Supported in part by NIH grants AI-13388 and AI-13484, NSF grant BMS-75-09513, National Foundation–March of Dimes grant 1-430, MUSC faculty research appropriations, and (to A.C.W.) American Cancer Society faculty research award 125. This is paper No. 175 from the Department of Basic and Clinical Immunology and Microbiology of the Medical College of South Carolina. Support to G.W.L.'s laboratory is by grants CA-08748 and AI-13528 from the National Cancer Institute.

10 November 1977; revised 15 February 1978

Crankcase Oils: Are They a Major Mutagenic Burden in the Aquatic Environment?

Abstract. *Fractions from used crankcase oil enriched in polyaromatic hydrocarbons induced revertant colonies in* Salmonella typhimurium *strain TA 98 when activated by rat or trout liver extracts. The mutagenic activity was not due to benzopyrene or benzanthracene. Fractions from various crude and refined petroleums were nonmutagenic. Among various petroleum hydrocarbons entering inland and coastal waters, used crankcase oils may represent a major mutagenic burden.*

There has been considerable speculation concerning the mutagenic or carcinogenic (*1*) potential of polycyclic aromatic hydrocarbons (PAH) from petroleum spills in the aquatic environment (*2, 3*). Petroleum contains a complex of aromatic and heterocyclic compounds including the classical carcinogens benzopyrene (BP) and benzanthracene (BA) (*4*); besides these aromatic hydrocarbons, evidence has been presented for other carcinogenic compounds in oil (*5*). There is increasing speculation that some marine animal tumors may have a pollutant etiology (*6*), and the increase in PAH in the aquatic environment has generated concern about possible long-term adverse effects on the health of aquatic organisms as well as human consumers of fish.

Aromatic hydrocarbon hydroxylases (AHH) are involved in the bioactivation of aromatics to mutagens in mammalian systems, and these enzymes are now known to occur in most marine organisms (*7, 8*). Screening of chemicals for mutagenic activity is commonly performed by a procedure developed by Ames *et al.* (*9*). In the work reported here we used this method to test for the

Table 1. Mutagenicity of used crankcase oil fractions toward *S. typhimurium* strain TA 98. The results are means of two experiments, each performed in duplicate. Student's *t*-test was used for statistical evaluation. Background was taken to be the number of spontaneous revertants generated in (i) the absence of hydrocarbons or (ii) the absence of supernatant from the 9000*g* rat or trout liver fractions. Oil irradiation was carried out with a long-wave ultraviolet lamp on a DMSO extract at a distance of 25 cm for 2 hours. When DMSO extracts of crankcase oil were chromatographed on silica gel thin-layer plates (Macherey-Nagel, GF 254) and developed with benzene, at least six bands were noted. The active fraction, 5, corresponds to the band between R_F 0.35 and 0.6. Fractions were eluted with methylene chloride and evaporated to dryness, and the residues were dissolved in DMSO. Both BP and BA have R_F values > 0.65. Aromatic hydrocarbon hydroxylase activity was induced in trout on exposure to petroleum. Enzyme assays were carried out with supernatants from the 9000*g* liver fractions as previously described (*16*). Fractions were prepared from three or four pooled fish livers. To assay AHH, alkali-extractable fluorescence was measured at excitation wavelengths of 395 and 520 nm. These wavelengths are specific for the fluorescent 3-OH and 9-OH BP derivatives. Specific activity increased from 4 to 5 units in liver homogenates from control fish to 40 to 50 units in those from fish exposed to petroleum.

Test conditions	His+ revertants per plate
Used oil	45
Used oil plus 9000*g* rat liver fraction	415*
Irradiated oil plus 9000*g* rat liver fraction	535*
Fraction 5 plus 9000*g* rat liver fraction	600*
Fractions 1, 2, 3, 4, and 6 plus 9000*g* rat liver fraction	60
Benzopyrene (5 μg) plus 9000*g* rat liver fraction	240*
Used oil plus 9000*g* uninduced trout liver fraction	79
Used oil plus 9000*g* induced trout liver fraction	400*
Rat or trout 9000*g* supernatant	30

*Significantly above background, $P < .01$.

0036-8075/78/0421-0329$00.50/0