may explain the nonidentity of HBsAg and HBeAg observed in gel diffusion analysis (*2, 3*).

Thus, electrophoresis of HBsAg-containing serums in polyacrylamide gel followed by functional assay of LDH isozymes provides a simple, sensitive, and rapid method of detecting the HBeAg by demonstration of an extra band of LDH-5ex. Chromatographic isolation of LDH-5 according to the empirical minicolumn procedure of Mercer (*14*) enhances the pattern and facilitates discrimination between LDH-5 and LDH-5ex. This assay may be valuable in laboratory diagnosis and prognosis of infectivity and chronicity of liver disease produced by infection with hepatitis B virus.

The present findings suggest that a complex is formed between LDH-5 and a component of HBsAg which alters the electrophoretic mobility to produce the LDH-5ex band. This raises the possibility that HBeAg may be a host component or a determinant of subtle conformational changes in LDH-5, but the exact nature of HBeAg is unknown. However, the clinical importance of HBeAg leads us to suggest that LDH-5ex may well serve as the hepatocyte target for cytotoxic lymphocytes causing hepatocellular injury.

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References and Notes

1. Proceedings of the National Research Council Symposium on Viral Hepatitis, *Am. J. Med. Sci.* **270**, 1 (1975).
2. L. O. Magnius and J. A. Epsmark, *J. Immunol.* **109**, 1017 (1972).
3. L. O. Magnius, A. Lindholm, P. Ludin, S. Iwarson, *J. Am. Med. Assoc.* **231**, 356 (1974).
4. In 1976 the World Health Organization Committee on Viral Hepatitis suggested the terms HBeAg and anti-HBe for the "e" antigen and antibody to e, respectively.
5. J. O. Nielson, O. Dietrichson, E. Juhl, *Lancet* **1974-II**, 913 (1974); S. V. Feinman, B. Berris, J. C. Sinclair, *ibid.* **II**, 1173 (1975); N. Eleftherious, H. C. Thomas, J. Heathcote, S. Sherlock, *ibid.*, p. 1171; E. Nordenfelt and L. Kjellen, *Int. Virol.* **5**, 225 (1975); N. E. Sheikh, I. L. Wolf, R. M. Galbraith, A. L. W. Eddleston, I. W. Dymock, R. Williams, *Br. Med. J.* **4**, 252 (1975); A. J. Zuckerman, *Nature (London)* **253**, 13 (1975); M. Imai, F. C. Tachibana, Y. Moritsugu, Y. Miyakawa, M. Mayumi, *Infect. Immun.* **14**, 631 (1976); V. J. McAuliffe, R. H. Purcell, G. L. Le Bouvier, *N. Engl. J. Med.* **294**, 779 (1976); S. H. Hindman, C. R. Gravelle, B. L. Murphy, D. W. Bradley, W. R. Budge, J. E. Maynard, *Ann. Int. Med.* **85**, 458 (1976); K. Okada, I. Kamiyama, M. Inomata, M. Imai, Y. Miyakawa, M. Mayumi, *N. Engl. J. Med.* **294**, 746 (1976); K. Takahashi, M. Imai, F. Tsuda, T. Takahashi, Y. Miyakawa, M. Mayumi, *J. Immunol.* **117**, 102 (1976); H. J. Alter, L. B. Seeff, P. M. Kaplan, V. J. McAuliffe, E. C. Wright, J. L. Gerin, R. H. Purcell, P. V. Holland, H. J. Zimmerman, *N. Engl. J. Med.* **295**, 909 (1976); E. Nordenfelt and M. Andrensandberg, *J. Infect. Dis.* **134**, 85 (1976); C. G. Trepo, L. O. Magnius, R. A. Schaefer, A. M. Prince, *Gastroenterology* **71**, 804 (1976); J. L. Smith, B. L. Murphy, M. O. Auslander, J. E. Maynard, S. S. Schalm, W. H. J. Summerskill, G. L. Gitnick, *ibid.*, p. 208; G. F. Grady and U.S. Cooperative Group Study, *Lancet* **1976-II**, 492 (1976); M. L. Tiku, G. M. Makhdoomi, K. R. Beutner, N. Nath, P. L. Ogra, *J. Pediatr.*, in press; K. C. Lam, M. J. Tong, J. Rakela, *Infect. Immun.* **16**, 403 (1977); M. J. Tong, D. Stevenson, I. Gordon, *J. Infect. Dis.*, in press.
6. L. O. Magnius, *Clin. Exp. Immunol.* **20**, 209 (1975).
7. A. R. Neurath and N. Strick, *Lancet* **1977-I**, 146 (1977); *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1702 (1977).
8. Although not completely characterized, two possible serotypes, e_1 and e_2, have been described by A. Williams and G. Le Bouvier [*Bibl. Haematol. (Basel)* **43**, 71 (1976)].
9. C. L. Markert and F. Moller, *Proc. Natl. Acad. Sci. U.S.A.* **6**, 753 (1959); N. O. Kaplan and S. White, *Ann. N.Y. Acad. Sci.* **103**, 835 (1963); A. Pesce, T. P. Fondy, F. Stolzenbach, F. Castillo, N. O. Kaplan, *J. Biol. Chem.* **242**, 2151 (1967).
10. J. Everse and N. O. Kaplan, *Adv. Enzymol. Relat. Areas Mol. Biol.* **37**, 61 (1973). For an up-to-date review of structure and function of lactate dehydrogenase, see C. L. Markert, Ed., *Isozymes* (Academic Press, New York, 1975), vols. 1 and 2.
11. S. R. Damle and R. V. Talavadekar, *Ind. J. Cancer Res.* **11**, 419 (1974); A. J. Baxi, J. P. Bapat, S. R. Damle, R. V. Talavadekar, R. M. Rajpal, J. K. Dave, *Vox Sang* **31**, 70 (1976).
12. Abnormal patterns of LDH isozymes have been reported as being due to complexing of pathological immunoglobulins with LDH [P. O. Ganrot, *Experientia* **23**, 593 (1967); L. P. Cawley, B. Minard, G. W. Byloma, M. Kelly, *Am. J. Clin. Pathol.* **50**, 606 (1968); J. Biewenga, *Clin. Chim. Acta* **40**, 407 (1972); M. Nagamine, *ibid.* **36**, 139 (1972); J. Biewenga, *ibid.* **76**, 149 (1977); D. W. Thomas, S. W. Rosen, R. Kahn, R. Temple, N. M. Papadopoulos, *Ann. Int. Med.* **81**, 434 (1974).]
13. P. Holland, G. Le Bouvier, J. Maynard, J. Mosley, A. Prince (N.Y.), and R. Purcell and V. McAuliffe tested six serums originally examined for e and anti-e by L. Magnius in order for us to select reference reagents for HBeAg and anti-HBe. The anti-HBe serum included both anti-e_1 and anti-e_2. Similar reagents from chimpanzees were received from H. Fields. Specific guinea pig antiserum to the *ay* subunit of HBsAg was obtained from the National Institute of Allergy and Infectious Diseases through the courtesy of R. H. Purcell. Specific antibody to the *ad* subunit of HBsAg was prepared in our own laboratory by immunization of guinea pigs against the polypeptide component (22,000 daltons) of *ad* antigen [D. L. Peterson, I. M. Roberts, G. N. Vyas and I. M. Roberts, *Vox Sang*, in press; (1977)]. Anti-HBc was procured from an HBsAg carrier whose IgG contained no anti-HBs but contained a high titer of anti-HBc that we used for a radioimmunoassay for anti-HBc [G. N. Vyas and I. M. Roberts, *Vox Sang*, in press; ———, D. L. Peterson, P. V. Holland, *J. Lab. Clin. Med.* **89**, 428 (1977)]. Polyvalent antiserum to human immunoglobulins prepared in goats was obtained from Meloy Laboratories. Specific antiserum to human LDH-5, when added to normal human serum, removed LDH-5 without affecting the remaining four isozymes. The antiserum was originally prepared and characterized by J. S. Nisselbaum and O. Bodansky, *J. Biol. Chem.* **236**, 401 (1961). The IgG from each antiserum was chromatographed in batches on diethylaminoethyl DEAE-A50 [D. M. Weir, *Experimental Immunochemistry* (Blackwell Scientific Publication, Oxford, 1973)]; The concentration of the IgG fraction from each of the antiserums ranged from 3 to 5 mg/ml.
14. D. W. Mercer, *Clin. Chem.* **21**, 1102 (1975).
15. This research was supported by NIH contract N01-3-HL-3000 and the Liver Center grant 1-P50-AM18520. We thank A. J. Baxi of the Blood Group Reference Center, Bombay, for discussions, J. S. Nisselbaum of the Sloan-Kettering Institute of Cancer Research for the well-characterized rabbit antiserum specific for human LDH-5, and R. Schmid and G. Brecher for critical review of this report.

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Group-Specific Component: Evidence for Two Subtypes of the Gc^1 Gene

Abstract. *A new method based on isofocusing electrophoresis in the study of the Gc (group-specific component) polymorphism, revealed differing electrophoretic patterns. These patterns can be explained by the existence of two codominant Gc^1 subtypes. This hypothesis is in accordance with several family studies. These subtypes are called Gc^{1F} and Gc^{1S}. Eight hundred samples were analyzed, including three different populations: Caucasoid (a western Pyrenean valley), African (Pygmy Bi-Aka), and Amerindian (Quechua-Aymara, from Bolivia). These two subtype phenotypes cannot be explored with the usual technique. They were present in each population sample studied.*

The Gc polymorphism is based on the existence of three electrophoretic phenotypes: Gc 1-1, Gc 2-1, and Gc 2-2. Additional variants of Gc^1 or Gc^2 gene are also observed (*1*). Different methods are described and commonly used (*2, 3*) to bring out this Gc polymorphism. Two autosomal and codominant Gc^1 and Gc^2

Table 1. Distribution of the Gc^{1S} and Gc^{1F} gene frequencies in the different samples studied. In this table the Gc^{Ab} variant present in the Pygmy sample is not included, nor are the Gc^2 and Gc^1 variants observed.

Country	Population	Gc^1 subtypes		χ^2	N
		Gc^{1F}	Gc^{1S}		
France	Pyrenean valley, L'Ouzom	0.077	0.512	0.659	290
Central Africa Empire	Pygmy	0.584	0.191	2.351	267
Bolivia	Quechua-Aymara	0.231	0.636	1.046	253

genes control the synthesis of the distinctive protein bands revealed by electrophoresis (*4*).

During the study of the Gc polymorphism in different population samples, we observed with the disc electrophoresis method (*5*) quantitative variations in the intensity of the protein bands, and also tenuous differences in the mobilities of the Gc^1 bands. Such patterns are confirmed by a cross immunoelectrophoresis (*6*).

The disc electrophoresis method was not sufficient to analyze such variations. We elaborated a new technique based on isofocusing electrophoresis with the use of a commercial ampholine solution LKB (*p*H range, 4 to 6) (*7*). Immunofixation was obtained with a monospecific antiserum to Gc (Behring or Atlantic antibodies). Each serum sample was studied simultaneously with disc and isofocusing electrophoresis. The two electrophoretic patterns obtained are shown in Fig. 1.

Isofocusing polyacrylamide plates revealed heterogeneity in the Gc^1 band zone: two sets of bands are shown (Fig. 2) which correspond to three different phenotypes that can only be explained by the existence of two Gc^1 subtypes. Two homozygous and one heterozygous phenotypes were present. The existence of two codominant and autosomal alleles was confirmed by family histories, and they justify such electrophoretic patterns. We called them Gc^{1F} and Gc^{1S} (1F, fast; 1S, slow).

In the Gc 1F-1F phenotype, the two protein bands present an equal intensity and a greater mobility than the ones corresponding to the Gc 1S-1S phenotype. The Gc 1F-1S phenotype is characterized by four electrophoretic band patterns. In this phenotype, the nonabsolute linearity of the *p*H gradient within the Gc focusing zone is responsible in some experiments for small space difference between the two anodic bands as compared to the cathodic bands. After immunofixation, minor bands were present in the anodic zone and correspond to immunological reacting material.

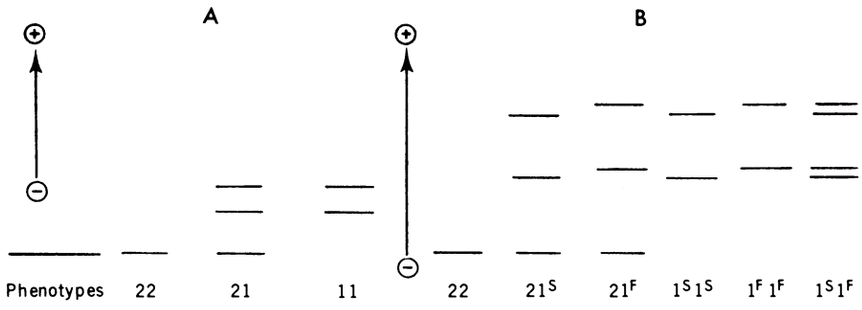

Fig. 1. Comparative mobilities of protein bands in disc electrophoresis (A) and immunofixation isofocusing electrophoresis (B).

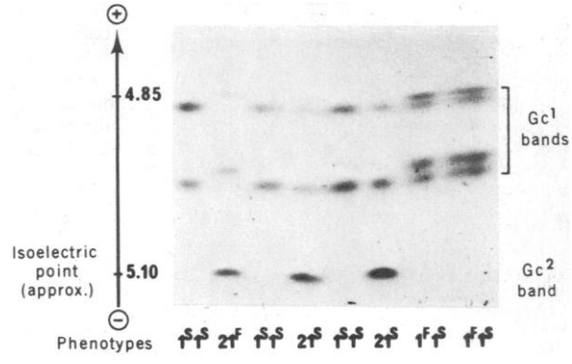

Fig. 2. Electrophoretic pattern corresponding to different phenotypes described after 4H30 migration on a 1-mm polyacrylamide gel at *p*H 4 to 6 in an ampholine solution.

The usual Gc 1-1 phenotype is represented by the following phenotypes: Gc 1F-1F, Gc 1F-1S, and Gc 1S-1S. The association of Gc^{1S} and Gc^{1F} subtypes with the Gc^2 gene provide two additional Gc 2-1F and Gc 2-1S phenotypes (Fig. 2).

Serum samples from three different populations were studied: a group from a western Pyrenean valley, a Pygmy sample (Bi-Aka), and an Amerindian tribe from Bolivia (*8*).

The method described revealed that the Pygmy population contained a variant of the Gc^2 gene, and that the Amerindian population contained a variant of the Gc^1 gene. The two subtypes Gc^{1S} and Gc^{1F} can be found in the three countries under study; their frequencies vary.

The isofocusing electrophoresis used revealed a new Gc polymorphism, which should prove useful in anthropological surveys and in disputed paternity cases. More data are needed on the geographical distribution of these subtypes and on their relation to the biological activity of the different phenotypes in the carrying of vitamin D (*2*).

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References and Notes

1. H. Cleve, *Isr. J. Med. Sci.* **9**, 1133 (1973).
2. S. P. Daiger, M. S. Schanfield, L. L. Cavalli-Sforza, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2076 (1975).
3. A. M. Johnson, H. Cleve, C. Alper, *Am. J. Hum. Genet.* **27**, 728 (1975).
4. J. Hirschfeld, *Acta Pathol. Microbiol. Scand.* **47**, 160 (1959).
5. F. D. Kitchin, *Proc. Soc Exp. Biol. Med.* **119**, 1153 (1965).
6. P. O. Ganrot, *Scand. J. Clin. Invest.* **29** (Suppl. 124), 39 (1972).
7. M. Viau, J. Constans, C. Bouissou, *Sci. Tools* **24** (2), 25 (1977).
8. We thank Dr. Jaeger for providing us serum samples from the Pygmy population and Dr. Y. Quilici for Amerindian samples. We are indebted to Prof. H. Cleve for comments in this study.

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