creased to a nadir of 29 mg percent at 30 minutes. Venous glucose rose and hunger ratings decreased toward the baseline over the next 60 minutes (D. A. Thompson, in preparation). These results confirm those reported by Janowitz and Ivy (*7*). In preliminary experiments, 2DG (50 mg/kg) was infused for 20 minutes into six healthy volunteers who rated their hunger highest at 120 minutes (a time when plasma glucose was maximally elevated). Both plasma glucose concentrations and hunger ratings remained elevated for at least another 60 minutes.

16. Category rating scales of subjective feelings consisted of seven categories for each attribute. Decreasing values for ratings of hunger or thirst represent increasing hunger or thirst with rating 1 referring to extreme hunger or thirst, 4 being neutral, and 7 referring to no desire to eat or drink even on request. Two scales appeared on each page, but related scales (such as hunger and fullness of stomach, thirst and dryness of mouth, and mood and vigor) were on different pages.
17. Subjects rated hunger, thirst, and perceived body temperature by marking a vertical line on a 300-mm horizontal line anchored in the center by a vertical line labeled "standard" to represent the subjects' feelings at the beginning of an experiment. Deviations in millimeters from the standard line in positive (increasing) or negative (decreasing) numbers were recorded.
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Hepatitis B "e" Antigen: An Apparent Association with Lactate Dehydrogenase Isozyme-5

Abstract. *Serums containing the "e" antigen of hepatitis B virus were subjected to electrophoresis in polyacrylamide gel. An extra band appeared in the lactate dehydrogenase isozyme pattern, but this band was undetectable in serums containing antibodies to the e antigenic determinant. Prior separation of the lactate dehydrogenase isozyme-5 fraction by chromatography of serum on minicolumns of diethylaminoethyl-Sephadex-A50 improved electrophoretic identification of the extra band. Neutralization with antibodies to the e antigen as well as by antibodies to the homologous d or y component of the hepatitis B surface antigen removed the extra band; antibodies to the lactate dehydrogenase isozyme-5 removed both the normal and the extra enzymatic band of isozyme-5. This feature of the e antigen provides an assay system for laboratory diagnosis of potential clinical usefulness and suggests its possible role in pathogenesis of hepatocellular injury.*

Immunologic and enzymatic markers of infection with the hepatitis B virus (HBV) include the hepatitis B surface antigen (HBsAg) with mutually exclusive *adw*, *ayw*, *adr*, and *ayr* subtypes, antibodies to HBsAg (anti-HBs), hepatitis B core antigen and antibodies (HBcAg and anti-HBc, respectively), and HBV specific DNA polymerase (*1*). Magnius and Epsmark (*2*) defined a new immunologic marker, the "e" and anti-e system, by gel diffusion analyses of precipitin reactions between various serums containing HBsAg, and suggested that the e antigen may be an indicator of contagiousness (*2, 3*). This marker has been designated HBeAg, and its antibodies, anti-HBe (*4*). Numerous investigators have found a positive correlation between the e antigen and DNA polymerase, Dane particle counts, infectivity, and chronicity of hepatitis (*5*), suggesting that the clinical outcome of the disease is more favorable in the presence of anti-HBe. The HBeAg has been characterized by Magnius (*6*) as a soluble protein distinct from HBsAg, with electrophoretic mobility in the fast gamma region and an estimated molecular weight of 300,000; Neurath and Strick (*7*) have postulated that HBeAg is a dimer of immunoglobulin G. Williams and Le Bouvier (*8*) have reported antigenic heterogeneity and thermolability of the HBeAg. We report here that HBeAg is associated with an isozyme of serum lactate dehydrogenase.

Serum lactate dehydrogenase (LDH, L-lactic acid NAD oxidoreductase, E.C. 1.1.1.27) consists of five isozymes (LDH-1, -2, -3, -4, and -5) of identical molecular weight but different charge, which permits their separation by electrophoresis; being the least negatively charged isozyme, LDH-5 separates in the gamma region (*9*). The LDH isozymes represent tetramers consisting of various combinations of two different polypeptide chains (M and H) each of 35,000 daltons (*9*). The isozyme LDH-5, composed of four noncovalently bound M chains, is the major component of the LDH synthesized by the liver cells (*10*). In serums of HBsAg positive individuals, Damle and co-workers (*11*) found an "anomalous" or extra band between LDH-4 and LDH-5, and also observed the removal of this anomalous band by anti-HBs (*11*). Complexing of immunoglobulins with LDH is known to produce abnormal isozyme patterns (*12*).

Because this extra band was present in all of the 90 HBsAg positive serums but in none of a series of HBsAg negative samples, it was suggested that this new finding might serve as a convenient method for detection of HBsAg (*11*). We confirmed this observation and further detected that this extra band is absent in serums of individuals containing anti-HBe. Moreover, this extra band was removed by immunochemical neutralization with specific antibodies against HBeAg, LDH-5, and the *d* or *y* subtypes of HBsAg. We designated this extra band as LDH-5ex.

The HBsAg was identified by a solid-phase radioimmunoassay (Ausria II, Abbott). Plasma units from apparently healthy carriers of HBsAg were obtained from blood banks in northern California and kept frozen at −20°C as a research

Table 1. HBsAg, LDH-5, and LDH-5ex of serum or plasma from hepatitis patients and asymptomatic HBsAg carriers who were accepted as blood donors.

Source of serum	Number of specimens	Number positive for		
		HBsAg	LDH-5	LDH-5ex
Acute non-B hepatitis	20	0	20	0
Acute hepatitis B	30	30	30	30
Asymptomatic HBsAg carriers with HBeAg	5	5	5	5
Asymptomatic HBsAg carriers with neither HBeAg nor anti-HBeAg	13	13	13	10
Asymptomatic HBsAg carriers with anti-HBe	14	14	14	0
Normal human donors	10	0	10	0

Table 2. Neutralizing capacity of antibodies against HBeAg, LDH-5, HBcAg, and IgG reacting with DEAE-Fr 1 isolated from serums showing the extra LDH isozyme band.

Neutralization with	LDH isozyme pattern after neutralization	
	LDH-5	LDH-5ex
Anti-HBe	+	−
Anti-LDH-5	−	−
Anti-HBc	+	+
Anti-IgG	+	+

resource since 1972 and 1973. Portions of these plasma units were tested for HBsAg subtypes, HBeAg, and anti-HBe as determined by gel diffusion analyses by Magnius *et al.* in 1974 (*3*). On the basis of results of gel diffusion analyses performed by seven different laboratories in the United States, serums containing HBeAg and anti-HBe from two asymptomatic healthy carriers of HBsAg were selected as reference reagents (*13*). For LDH isozyme analysis portions (100 μl) of test serum or chromatographic fractions were mixed with 100 μl of 40 percent aqueous solution of sucrose and sandwiched in a 5.8 percent acrylamide gel. Electrophoresis was conducted with 0.005*M* tris glycine buffer, *p*H 8.3, and a current of 5 ma per gel for a period of 3 hours at 4°C. The gel was stained for 15 minutes in a solution containing 1.0 percent lithium lactate, 0.04 percent nitro blue tetrazolium, 0.1 percent nicotinamide adenine dinucleotide (NAD), and 0.005 percent phenazine methosulfate in 0.1*M* tris buffer, *p*H 8.0. Extra stain was removed with distilled water, followed by 5.0 percent acetic acid. The electrophoretic pattern of LDH isozymes of serums of normal and HBeAg-containing serums is shown in Fig. 1. Neutralization experiments were performed by mixing test serum or isolated LDH fractions with immunoglobulin G (IgG) isolated from specific antiserums (*13*); incubation for 30 minutes at ambient temperature (23° to 24°C) and analyses of the mixture for LDH isozymes in polyacrylamide gel removed the reactive isozyme band.

Five HBeAg positive units from asymptomatic HBsAg carriers exhibited LDH-5ex that could be neutralized by anti-HBe; by contrast, none of the 14 specimens containing anti-HBe showed LDH-5ex (Table 1). A randomly selected group of 13 plasma specimens from HBsAg carriers, without HBeAg or anti-HBe demonstrable by gel diffusion, showed LDH-5ex activity that could be neutralized by anti-HBe in ten of the specimens (Table 1); the remaining three specimens contained neither the LDH-5ex nor anti-HBe, as checked by their failure to remove the LDH-5ex band from a reference HBeAg serum. Specimens obtained from patients with acute hepatitis included 30 serums positive for HBsAg and 20 negative for HBsAg (Table 1). The LDH-5ex observed in all of the 30 positive serum specimens was removed by reaction with anti-HBe. Therefore, observation of LDH-5ex was considered a more sensitive means of detecting HBeAg than the immunoprecipitation in gel.

Because isolated LDH isozymes reveal higher LDH activity than the whole serum (*14*), chromatographic separation of LDH isozymes was performed on five representative specimens from each of the groups of serums in Table 1 by a modification of the chromatographic method of Mercer (*14*). A disposable plastic column was packed with a bed volume (7 by 0.6 cm in diameter) of about 2.0 ml of DEAE-A50 in 0.05*M* tris and 0.1*M* sodium chloride, *p*H 8.0. A portion (1.0 ml) of serum was passed through the column and the eluate was discarded. Five consecutive 1.0-ml fractions were then obtained by stepwise elution with 1.0 ml each of the packing buffer. Subsequently, four additional fractions were eluted with the same buffer but with an increased molarity of 0.2*M* NaCl. All nine fractions, serially labeled DEAE-Fr 1 through Fr 9, were analyzed for HBsAg and LDH isozymes. DEAE-Fr 1 contained all the HBsAg activity and all the LDH-5 isozyme activity. DEAE-Fr 1 from normal serums or serums with anti-HBe revealed one band of LDH-5, but two discrete bands were seen when the original serums contained the LDH-5ex (Fig. 1); the LDH-5ex band detectable in either the serum or the plasma was more clearly demonstrable in DEAE-Fr 1 than in the original specimen. The fast-migrating band of LDH-5ex was removed by anti-HBe. DEAE-Fr 2 through Fr 5 contained LDH-4- and LDH-3, whereas Fr 6 to Fr 9 contained LDH-3, -2, and -1, which conforms to the original description of Mercer (*14*).

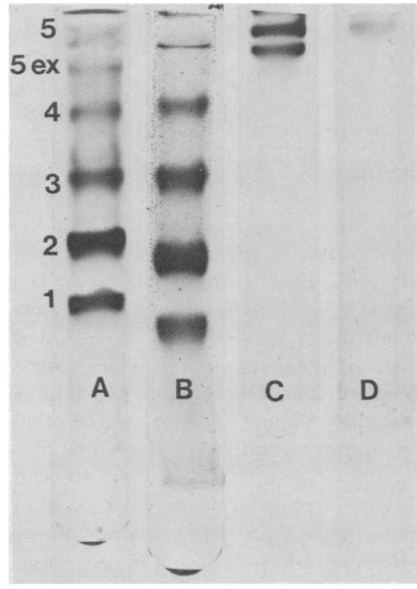

Fig. 1. Electrophoresis of serums or DEAE fractions in polyacrylamide gels showing LDH isozyme patterns. Gel A shows an extra band of LDH-5ex between LDH-4 and LDH-5 in a serum containing HBsAg and HBeAg; gel B shows LDH isozyme pattern of normal human serum; gel C shows LDH-5 and LDH-5ex in DEAE-Fr 1 from serum in gel A; and gel D shows LDH-5 in DEAE-Fr 1 from serum in gel B. Patterns of whole serum or DEAE-Fr 1 of the serum containing anti-HBe were identical with patterns in B or D, respectively; so also was the pattern obtained after neutralization reactions with anti-HBe or anti-HBs that specifically removed LDH-5ex.

Table 3. Removal of LDH-5ex by neutralization reaction with antiserum to *ad* or to *ay* showing homologous *d* or *y* component of HBsAg (without the group specific *a* determinant) in association with LDH-5 in DEAE-Fr 1 derived from serums with *adw* or *ayw* subtypes of HBsAg. The normal band of LDH-5 remained unaffected by reaction with antibodies.

DEAE-Fr 1 of serum with HBsAg subtype	Neutralization by antibodies to	
	ad	*ay*
adw	+	−
adw	+	−
ayw	−	+
ayw	−	+
Normal serum	−	−

In Table 2 the data on the serologic specificity of the HBeAg associated with LDH-5 is demonstrated by the neutralization of DEAE-Fr 1 with specific anti-HBe which removes LDH-5ex, and with antibody to LDH-5 which removes both the LDH-5 and LDH-5ex bands. Neither anti-HBc nor antibody to IgG would remove LDH-5 or LDH-5ex. The results suggest that LDH-5ex contains antigenic determinants for LDH-5 and HBeAg, but not for HBcAg or IgG.

In confirming the observation of neutralization of the extra band with anti-HBs (*11*), we found only homologous neutralization of LDH-5ex by antibody to the *ad* or *ay* subunit, depending on whether the original serum was derived from the *adw* or *ayw* subtypes of HBsAg, respectively. The serologic dissociation of *d* or *y* component from the common *a* determinant of HBsAg subtypes *adw* and *ayw*, respectively, is illustrated by the results in Table 3. This biologic dissection of the *d* or the *y* determinant from the common *a* determinant of HBsAg in association with LDH-5ex

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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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