TABLE II. GABA Levels in Brain of Mice Treated with L-Glutamic Acid-γ-Hydrazide or Pyridoxal Phosphate-γ-Glutamyl Hydrazone.

Treatment	Control	Treated	% Change
L-glutamic acid-γ-hydrazide (2 g/kg 3 hr before sacrifice)	2.74 ± .31 (8)	10.01 ± .33* (7)	+265
Pyridoxal phosphate-γ-glutamyl hydrazone (80 mg/kg 27 min before sacrifice)	3.56 ± .10 (4)	2.34 ± .09* (6)	— 34.3

The values are μmoles/g ± S.E.M. Number of animals in parentheses.
* $p < .001$ (difference from control values).

hydrazone, it is suggested that the rate of GABA formation, independently of its total concentration, is probably a factor in the production of some kinds of convulsions. It is also suggested that the site of GABA formation in brain appears to be a critical one, and that this critical site might be the synaptic cleft.

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Marburg Immunologic Reactivity of Haptoglobin: Evidence for a Beta-Chain Mutation.* (32407)

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The presence of several atypical types of human serum haptoglobin such as Hp 2-1 Johnson(1), 1-P, 2-P, 1-H, 2-L(2), Hp Ab (3) and Hp 2-1 D(4) have been reported by several investigators. These atypical types can be easily differentiated from the common three phenotypes, *i.e.*, 1-1, 2-1 and 2-2, by ordinary starch gel electrophoresis after addition of hemoglobin.

In addition, the existence of immunologically atypical haptoglobins which show normal starch gel electrophoretic patterns was also recognized by Korngold(5) and Shim and Bearn(6). The nature of these atypical haptoglobin molecules has not been clarified.

Haptoglobin Marburg (Hp Mb), thus far,

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is a unique type of haptoglobin which shows both atypical patterns on ordinary starch gel electrophoresis and also an atypical immunological reaction(7-10). In the present study, we have obtained immunological evidence that haptoglobin Marburg is a beta-chain mutant with abnormal hemoglobin binding.

Materials and methods. Sera of normal 2-1 type and 2-1 Marburg were kept frozen until immediately before use. Immunoelectrophoresis was carried out by the method of Grabar and Williams(11). The double gel diffusion technique was performed by the method of Ouchterlony(12). The specific rabbit antisera to Hp 2-2, and to isolated beta-2-2 and alpha-2-2 chains were those used previously by Shim and others(13,14).

Results. When haptoglobin Marburg was tested against anti-Hp 2-2 serum the reaction was indistinguishable from that of common Hp 2-1. Spur formation was seen between free Hp Mb and its complex with hemoglobin, between 2-1 Mb-Hb complex and 1-1 Hp-Hb complex as well as between 2-1 Mb and Hp 1-1 (Fig. 1 and 2). When an antiserum specific for normal 2-2 chains was used, haptoglobin Marburg, free or complexed with hemoglobin, did not differ from

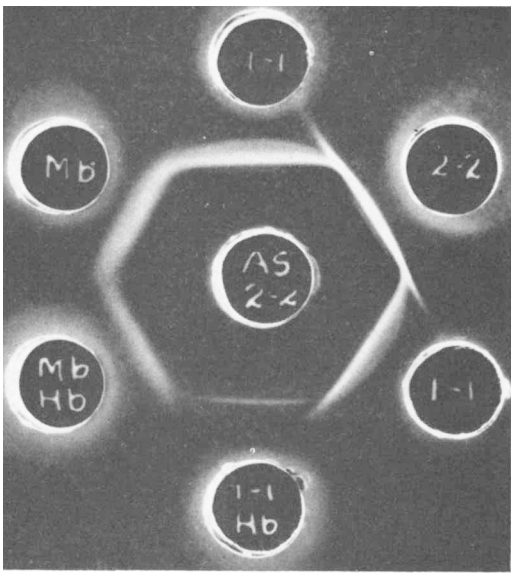

FIG. 1. Immunological reaction of haptoglobin Marburg in double diffusion studies in agar. Spur between free Mb and Mb-Hb complex is apparent even though weak. The spur between free Mb and free 1-1 is not clear-cut in the illustration.

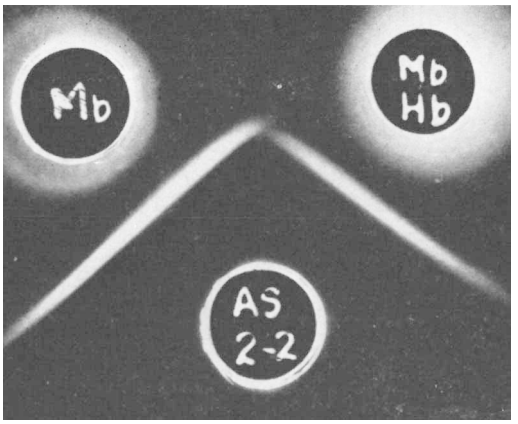

FIG. 2. Spur formation between free Mb and Mb-Hb complex. The spur is clearer than in Fig. 1.

normal Hp 2-1 in its reaction (not illustrated). However, the reaction of Hp 2-1 Mb against antiserum specific for the beta-chain was clearly different from that of normal Hp 2-1 (Fig. 3). As previously reported

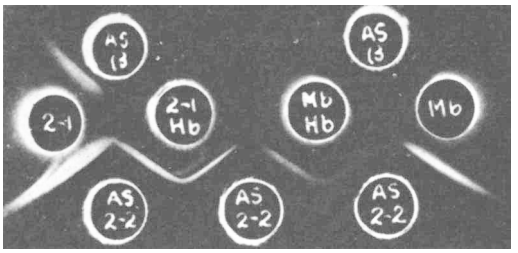

FIG. 3. Comparison of immunological reaction of common 2-1 Hp-Hb and 2-1 Mb-Hb complexes against anti-beta-chain serum. Common 2-1 Hp-Hb complex did not react with anti-beta-chain serum, whereas 2-1 Mb-Hb complex reacted.

by Shim and others(13,14) normal Hp 2-1 complexed with hemoglobin did not react against anti-beta chain serum. On the other hand, the hemoglobin complexed variant 2-1 Mb still reacted against the anti-beta chain serum. On immunoelectrophoresis Hp 2-1 Mb showed a precipitin line which extended further towards the anode than normal Hp 2-1, both free and complexed with hemoglobin, confirming previous observations by Cleve and Deicher(10).

Discussion. Haptoglobin molecules of all genetic types are composed of two kinds of polypeptide chains, alpha- and beta-chains (6,15,16). Shim and others(13,14) demonstrated that haptoglobin binds with hemoglobin at the beta-chain by immunologic tech-

TABLE I. Immunological Reactivities of Haptoglobin Marburg and Comparison of Specificities of Two Antisera.

Reaction	Shim, *et al*	Beuing, *et al*	Remarks
1. Spur between Mb and Mb-Hb	+ (Mb-Hb deficient)	—	due to coverage of B_1 determinants by Hb in normal components of heterozygous Hp Mb molecules
2. Spur between 2-1 Hp-Hb and Mb-Hb	—	++ (2-1 Hp-Hb deficient)	due to B_2 determinants which is inaccessible in normal 2-1 complex but accessible in Mb-Hb complex
3. Spur between 1-1 Hp-Hb and Mb-Hb	+ (1-1 Hp-Hb deficient)	++ (1-1 Hp-Hb deficient)	due to S determinants in case of Shim's antiserum or similar to reaction 2 in case of Beuing's antiserum
4. Spur between 2-1 and Mb	—	—	
5. Spur between 1-1 and Mb	+ (1-1 deficient)	—	due to genetic type specific S determinants
6. Spur between common free Hp and its Hb-complex (regardless of genetic type)	++ (Hb complex deficient)	++ (Hb-complex deficient)	due to coverage of B_1 (Shim) or inaccessibility of B_2 determinants (Beuing)
7. Korngold type spur	+++	—	due to type specific S determinants

* See text on S, B_1 and B_2 determinants.

niques, and it was later confirmed by Gordon and Bearn(17) by direct measurement of the hemoglobin binding capacity of the isolated beta-chain. In addition, several workers showed that some antigenic determinants of the haptoglobin molecule could be covered by binding hemoglobin(7,8,13,14,18). Therefore, the fact that hemoglobin complexed 2-1 Mb reacted against anti-beta-chain serum, whereas hemoglobin complexed normal 2-1 type did not, indicates that haptoglobin Marburg is a beta-chain mutant affecting the hemoglobin binding mechanism.

Atypical immunological reactivities of haptoglobin Marburg have been described elsewhere by Beuing and others(7-10). They used commercial horse antisera prepared against pooled normal human sera (Pasteur AS No. 223). With this antiserum, they observed spur formation between 2-1 Mb-Hb complex and normal 2-1 Hp-Hb complex whereas the specific rabbit anti-Hp 2-2 serum used in our study showed a reaction of identity. The different specificities of the two antisera have been summarized in Table I.

The antigenic determinants observed in the haptoglobin molecule can be classified into 2 categories, one group which is specific for the genetic type and shows a spur between Hp 2-2 and 1-1 as first described by Korngold (reaction 7 in Table I, S determinants) and the other group common to all 3 genetic types, C determinants. The common determinants C can be subclassified further into 2 subgroups, *i.e.*, A determinants which have no relation to hemoglobin binding and B determinants related to hemoglobin binding. Spurs in reaction 1 and reaction 2 listed in Table I are apparently due to different antigenic determinants. B determinants can therefore be further divided into 2 types, *i.e.*, B_1 determinants which showed a spur between free Hp Mb and Mb-Hb complex (reaction 1) and B_2 determinants which showed a spur between 2-1 Hp-Hb complex and Mb-Hb complex (reaction 2).

The spur in reaction 1 might be due to the covering by hemoglobin of some antigenic determinants of normal beta-chain present in heterozygous 2-1 Mb molecules. On the contrary, Beuing and others' antiserum showed a spur between Mb-Hb and normal 2-1 Hp (reaction 2 in Table I). This indicates that their antiserum does contain anti-

bodies against certain determinants which become inaccessible in normal haptoglobin on hemoglobin binding (B_2 determinants). These suggest that certain conformational changes occur in normal haptoglobin when binding hemoglobin in the region designated B_2 which make these determinants inaccessible. However, the B_2 determinants in haptoglobin Marburg molecule are still accessible even in the presence of excess hemoglobin. Therefore, the atypical immunologic properties of haptoglobin Marburg might be due to the alteration of hemoglobin binding sites on the mutant beta-chain, or alternatively, to an indirect effect on the conformation of haptoglobin Marburg by an amino acid substitution not involved in the binding site.

Recent investigations of B chain structure (19) showed that the beta-chains of all 3 genetic types are identical. Therefore, the S determinanats may reside in the alpha-2-2 chain, or be due to the polymeric configuration. The A determinants may be present in the alpha-chain or in the part of the beta-chain not concerned with hemoglobin binding. The B determinants should be present on the beta-chain.

Summary. The immunological reactivity of haptoglobin Marburg was examined with specific rabbit anti-Hp 2-2, anti-alpha-2-2 chain and anti-beta-chain sera. The anti-beta-chain serum indicated differences from normal Hp 2-1 and it was concluded that the beta-chain of haptoglobin Marburg shows anomalous hemoglobin binding. In addition, a comparison of the specificities of the present rabbit anti-Hp serum with a horse antiserum used by Beuing and others(7,8,10), enabled us to classify the antigenic determinants of the haptoglobin molecule into several subgroups.

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