Since clinical signs of upper respiratory infections in dogs vary from a slight rhinitis to severe tracheobronchitis which at times progresses to bronchopneumonia, it is possible that the primary viral lesions in the nasal, bronchial, and tracheal mucosae predispose the affected animal to secondary bacterial infections.

Summary. The isolation of herpescanis virus from the respiratory tract of naturally infected dogs is reported. Several animals from which the virus was isolated exhibited the characteristic symptoms of canine tracheobronchitis while others were not clinically ill.

Except for differences in *in vivo* and *in vitro* cytopathogenicity, the virus possesses similar physical, chemical, and morphological characteristics of the herpes group of viruses. It was positively identified by serologic methods as herpescanis virus.

Experimental reproduction of the respiratory disease was observed following intranasal inoculation of susceptible dogs, while inoculated pups died of acute hemorrhagic disease following intraperitoneal inoculation.

The pathogenicity of herpescanis virus is evaluated on the basis of our studies and those of other workers.

We wish to express our sincere thanks to Miss E. C. Adams of the Department of Pathology, Harvard Medical School for preparation of the electron micrographs and to Dr. L. E. Carmichael of the New York State Virus Research Institute for supplying us with herpescanis antiserum.

1. Karpas, A., King, N. W., and Garcia, F. G., Bacteriol. Proc. 157 (1967).

2. Andrewes, C. H. and Horstman, D. M., J. Gen. Microbiol. 3, 290 (1949).

3. Ben-Porat, T. and Kaplan, A. S., Virology 16, 261 (1962).

4. Carmichael, L. E., Squire, R. A., Krook, L., Am. J. Vet. Res. 26, 803 (1965).

5. Wildy, P., Russel, W. C., and Horne, R. W., Virology 12, 204 (1960).

6. Carmichael, L. E., Strandberg, J. D., Burnes, F. D., Proc. Soc. Exptl. Biol. Med. 120, 644 (1965).

7. Stewart, S. E., David-Ferreira, J., Lovelace, E., Landon, J., and Stock, N., Science 148, 1341 (1965).

8. Archibald, J., in "Canine Medicine," 2nd ed., p. 161. American Veterinary Publication Incorporation, (1959).

9. Spretzel, R. Q., Huxsoll, D. L., McConnell, S. J., Binn, L. N., and Yager, R. H., Proc. Soc. Exptl. Biol. Med. 120, 651 (1965).

Received April 3, 1967. P.S.E.B.M., 1968, Vol. 127.

An Improved Method of Preparing Haptoglobin Polypeptide Chains Using Guanidine Hydrochloride* (32619)

SIAMON GORDON, HARTWIG CLEVE, AND ALEXANDER G. BEARN
Rockefeller University, New York, N.Y.

Haptoglobin is an α_2 serum protein which has the property of binding hemoglobin stoichiometrically. Genetic studies of this protein in man have revealed a striking polymorphism of three main phenotypes, viz. haptoglobins 1-1, 2-2 and 2-1 (1). All 3 genetic types consist of two kinds of polypeptide chain linked by interchain disulfide bonds, a larger β and a smaller α chain[1] (2). In the case of haptoglobin 1-1 chain fractionation has been accomplished after reductive cleavage and a tentative model has been proposed for this molecule (3). Studies of haptoglobin 2-2 and 2-1 have not yet revealed the exact arrangement of β and α chains in these phenotypes.

Chemical studies of the α chains of all 3 types of haptoglobin have been in progress for several years (4,5). There has been little detailed study of the β chains, although there is evidence that isolated β chains prepared under certain conditions are still capable of binding hemoglobin, whereas isolated α chains are inactive (6). One difficulty has been the

* This investigation was supported in part by U. S. Public Health Service Grant No. AM01542-10.

[1] Abbreviations used in this paper: haptoglobin—hp; chains of each common type of hp denoted by subscript, e. g., $\beta 2\text{-}2$; $\alpha 2\text{-}2$; hemoglobin—hb.

preparation of purified β chains of all 3 types in good yield and suitable for further study, i.e., extensively denatured for structural analysis or, alternatively, still capable of binding hemoglobin.

To achieve these aims we have reduced and alkylated haptoglobins of all 3 types in the presence or absence of guanidine followed by fractionation in all cases in the presence of guanidine. Some of the properties of the resulting chains have been studied and will be reported in this paper.

Materials and Methods. 1. Purification. Fresh frozen unhemolyzed plasma was obtained from the New York Blood Center and in the case of all 3 common types of haptoglobin chromatographed on DEAE cellulose using gradient elution conditions described elsewhere (6). The haptoglobin-rich material obtained in this way was concentrated by ultrafiltration and subjected to Pevikon[2] block zone electrophoresis using 0.1 *M* barbital buffer, pH 8.6 (7). The α_2 globulin fraction was further purified by column chromatography on Sephadex equilibrated with a 0.05 *M* Tris HCl solution, pH 7.7. A different kind of Sephadex was used for each type of haptoglobin. Haptoglobin 1-1, mol. wt. 100,000 (8), enters the gel particles of G-200 so that this Sephadex could be used to remove both larger and smaller unwanted proteins. The material eluted in the void volume was therefore discarded and the second peak concentrated. Haptoglobins 2-2 and 2-1 consist of a series of polymers varying in molecular weight from 100,000 to several hundred thousand. To obtain all polymers it was necessary to use G-100 for hp 2-2 and G-75 for hp 2-1, with the haptoglobin in each case eluted within the void volume. Smaller contaminating proteins, e.g., albumin, could thus be separated from the haptoglobin. In all cases electrophoretically pure haptoglobin could be obtained. Benzidine staining after starch gel electrophoresis of each preparation of haptoglobin mixed with hemoglobin showed that this haptoglobin retained the ability to bind hemoglobin. About 150 mg of pure haptoglobin could be prepared in this way from 200 ml of plasma.

2. Purification of guanidine-HCl. Guanidine hydrochloride, Lot 44A, 749, was purchased from Eastman Organic Chemicals Co. Solutions of 5 *M* guanidine hydrochloride, 0.05 *M* Tris and pH 7.75 were prepared as follows: 1 kg of guanidine hydrochloride was dissolved in about 2 liters of twice distilled water. Activated charcoal was used to absorb impurities until the optical density at 280 mμ was less than 0.060. The molarity of the solution was determined by refractive index measurements using an Abbé refractometer and the data of Kielley and Harrington (9). The solutions at this stage were approximately 6–7 *M* and pH 4 and could then be adjusted to the desired composition with 2 *M* Tris and *N* HCl.

3. Reduction and alkylation. (a) For extensive reduction and denaturation the following procedure was used. Fifty mg of purified haptoglobin, at a protein concentration of about 10 mg per ml, were dialyzed overnight against 5 *M* guanidine-HCl, 0.25 *M* Tris, pH 8.2, at 4°C. After centrifugation, 0.2 *M* mercaptoethanol (Matheson, Coleman, and Bell) was used for 24 hours at room temperature to achieve extensive reduction. Alkylation was performed with 0.4 *M* iodoacetamide (Mann Research Laboratories) for half an hour at 4°C and in the dark. The pH remained alkaline to phenol red throughout this time. The sample was then dialysed at 4°C against two changes of 5 *M* guanidine-HCl, 0.05 *M* Tris, pH 7.75, with the buffer present in hundredfold excess. Since α1-1 chains have a molecular weight of 8900 (5) 18/32 Visking dialysis membrane was used to avoid losses during dialysis of all preparations containing chains of this size.

(b) To achieve milder reduction and retain binding activity there was no contact with guanidine until after reduction and alkylation had both been completed. Fifty mg of material at 10 mg per ml and in 0.05 *M* Tris HCl buffer, pH 8.2, were reduced for 1 hour at room temperature using 0.2 *M* mercaptoethanol and then alkylated with 0.24 *M* iodoacetamide for half an hour at 4°C. Excess alkylating reagent was removed by dialysis at 4°C, for 4 hours, against 2 changes of normal

[2] Pevikon, a polyvinyl chloride-acetate copolymer, was supplied by Superfosfat A. B., Stockholm, Sweden.

saline in hundredfold excess. The same precautions were taken as in (a) to control pH, prevent light exposure and avoid losses during dialysis.

4. Preparation of Sephadex G-200 in 5 M guanidine HCl, 0.05 M Tris, pH 7.75. Sephadex G-200 (Pharmacia) was sieved and the 100–200 micron fraction soaked in 5 *M* guanidine HCl, 0.05 *M* Tris, pH 7.75, for a week, to ensure complete swelling of the beads. After several washes with the same buffer the gel was freed of bubbles and packed in a column 33 inches × 1 inch, designed for upward flow. By using the lowest possible pump pressure flow rates of 8–10 ml per hour at 4°C could be obtained. Such a column could be used for several experiments at a reasonable flow rate.

5. Isolation of chains. The reduced and alkylated sample in both types of preparation was further dialysed at 4° against 5 *M* guanidine HCl, 005 *M* Tris, pH 7.75, for 16 hours after extensive reduction and for 4 hours after milder reduction. After centrifugation the sample was applied to the bottom of the column and eluted by upward flow using the same buffer. Four-ml fractions were collected and the absorbancy measured at 280 mμ. Such a separation could be completed in 2 days. The further handling of the chains differed for the two types of preparation. The fully reduced material was dialyzed for 3 days against 6 changes of 5% formic acid at 4°C and then freeze-dried. The more mildly reduced material was dialyzed against normal saline in similar fashion and concentrated by freeze drying or by ultrafiltration using 8/32 Visking dialysis tubing for β chains and 23/32 Visking tubing for α chains. The chains obtained after extensive reduction were dissolved in aqueous buffers with difficulty unless urea was added. The chains obtained after milder reduction dissolved readily in aqueous buffers, although some precipitate had to be spun off, especially in the case of $\alpha_{2\text{-}2}$ chains.

6. Urea formate starch gel electrophoresis was used to estimate the purity of the chains (10).

7. Amino acid analysis was performed in duplicate on 0.06-mg samples of extensively reduced β chains according to the method of Bowman and Barnet(11).

8. Binding studies. A 10% solution of hemoglobin was prepared according to the method described by Muller(12).

Peroxidase activity was assayed by the spectrophotometric method of Tarukoski(13). A 0.15% sodium chloride solution was used as a diluent for test samples. The extensively reduced material did not dissolve readily and was centrifuged to remove undissolved material before performing the assay.

9. Protein estimations were made using the Lowry method(14).

10. Immunological Studies. Specific precipitation by double diffusion was performed in 1% agarose in 1/15 *M* phosphate buffer, pH 7(15). Twenty μl samples of material at 1 mg/ml were used initially. When no line of precipitation was found the experiment was repeated using 100 μl of sample. Intrawell absorption was performed by allowing 20 μl of test material at 3 mg/ml to diffuse completely out of the well before filling it with antiserum and testing against normal hp 2-2.

To study the binding of hemoglobin by means of gel diffusion, the test sample at 1 mg/ml was fully saturated with hemoglobin and compared with the same material without any hemoglobin.

Antiserum specific for haptoglobin was prepared as follows: purified haptoglobin 2-2 (10 mg/ml) was emulsified with an equal volume of Freund's adjuvants and 1 ml of the emulsion injected subcutaneously into a Chinchilla rabbit. One ml boosters of the same haptoglobin without adjuvant was given at intervals of 1 month and 1 week. The antiserum obtained was absorbed with anhaptoglobinemic human serum and shown to be specific for haptoglobin by immunoelectrophoresis(16), testing the antiserum against normal human serum in the presence, as well as in the absence, of hemoglobin.

11. Purified human group specific protein (Gc) was provided by Dr. Barbara Bowman.

Results. The extent of reduction was estimated by amino acid analysis. When reduction was performed in the presence of guanidine there was no residual half-cystine, all having been converted to *S*-carboxymethyl

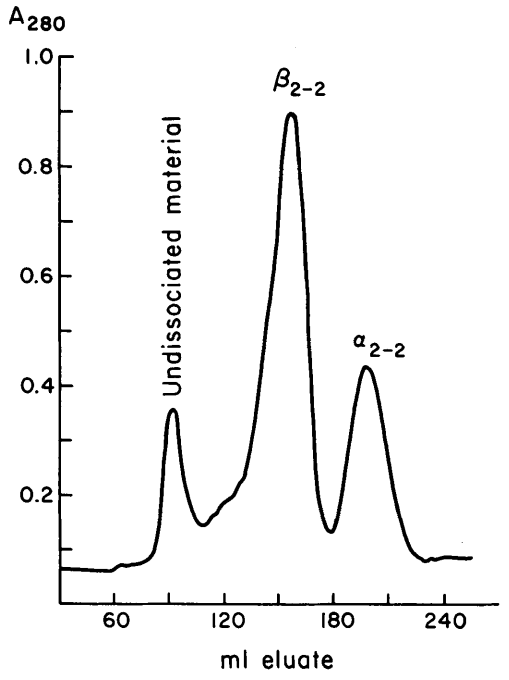

FIG. 1. Haptoglobin 2-2, reduced and alkylated in the absence of guanidine, fractionated on Sephadex G-200 in 5 *M* guanidine HCl, 0.05 *M* Tris, pH 7.75.

cysteine. When guanidine was absent during reductive cleavage 6 out of the 20 moles of half-cystine present per mole of protein were recovered as half-cystine. This corresponds to 3 unreduced disulfide bonds.

2. *Separation of chains. (a) Milder reduction.* An elution curve of haptoglobin 2–2, reduced and alkylated in the absence of guanidine and fractionated on Sephadex G-200 in guanidine, is shown in Fig. 1. The $\alpha_{2\text{-}2}$ chains were well separated from the $\beta_{2\text{-}2}$ chains and the yield of $\alpha_{2\text{-}2}$ chain relative to that of $\beta_{2\text{-}2}$ chain was 35%, i.e., complete when compared with the full theoretical yield obtainable. The undissociated material was eluted within the void volume. In the case of hp 1-1, the undissociated material was not as well separated from the $\beta_{1\text{-}1}$ peak and the recovery of $\alpha_{1\text{-}1}$ chain relative to $\beta_{1\text{-}1}$ was 14% instead of the theoretical complete yield of 18%.

(b) Extensive reduction. With all types of haptoglobin good separation and full yields of chains could be obtained. The peak of undissociated material was considerably smaller than in preparations which had been more mildly reduced. The $\alpha_{2\text{-}1}$ chains of haptoglobin 2-1 were recovered in a yield of 27% relative to that of $\beta_{2\text{-}1}$ chains, a result consistent with an equimolar ratio of α_2 and α_1 chains. Some separation of α_2 and α_1 chains was achieved.

3. *Electrophoresis in urea formate gels* showed that chains isolated after extensive reduction were pure (Fig. 2). Chains prepared by the milder method (not illustrated) were pure in the case of hp 2-2. Haptoglobin 1-1 α chains made by the milder method were likewise pure, but some slower moving material contaminated the $\beta_{1\text{-}1}$ preparation.

4. *Chain size estimation.* The molecular weights of $\alpha_{1\text{-}1}$ (8900) and $\alpha_{2\text{-}2}$ (17,300) are known (5), but no estimate of β chain molecular weight is available except indirectly from that of the whole hp 1-1 molecule. The elution positions of different chain preparations, as well as that of purified Gc protein (mol. wt. 51,000), were determined on Sephadex G-200 in 5 *M* guanidine. In Fig. 3 the known molecular weights for Gc $\alpha_{1\text{-}1}$ and $\alpha_{2\text{-}2}$ are plotted against the difference, for each chain or protein, between its elution volume and the total void volume of the column. A straight line was obtained. The β chain elution volumes

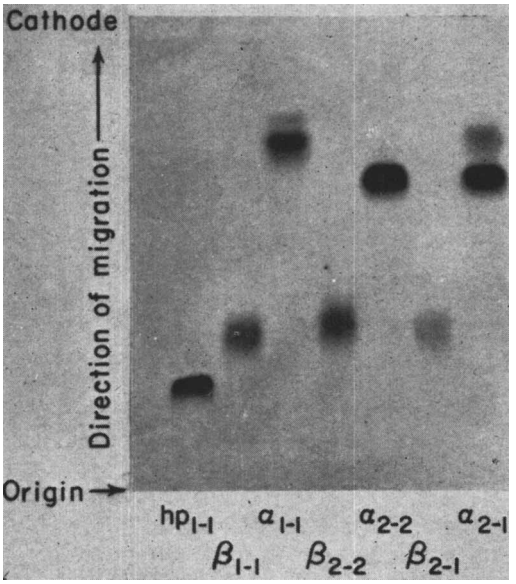

FIG. 2. Urea formate strach gel electrophoresis after extensive reduction.

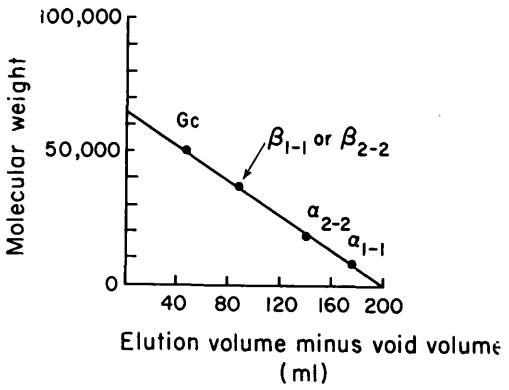

FIG. 3. Estimation of size of β chains using Sephadex G-200 in 5 *M* guanidine HCl 0.0 *M* Tris, pH 7.75.

suggest a molecular weight of about 38,000 for both β_{2-2} and β_{1-1} chains. The exclusion volume of Sephadex G-200 in 5 *M* guanidine HCl 0.05 *M* Tris, pH 7.75, corresponds to a molecular weight of about 64,000.

5. The amino acid analysis of β chains obtained after extensive reduction is listed in Table I. The absence of half-cystine indicates that reduction had been complete. The β chains of each common genotype show a remarkable similarity in composition. The relatively high proline content is also worthy of note.

TABLE I. Haptoglobin β Chain Amino Acid Analysis.[a]

Amino acid	1-1	2-2	2-1
Lysine	25	26	26
Histidine	11	11	11
Arginine	5	7	5
Carboxymethylcysteine	5	5	5
Aspartic Acid	31	33	33
Threonine	21	21	20
Serine	20	18	16
Glutamic Acid	33	33	33
Proline	12	15	15
Glycine	24	24	24
Alanine	25	23	25
Half-Cystine	—	—	—
Valine	31	29	31
Methionine	4	5	5
Isoleucine	15	13	16
Leucine	27	27	27
Tyrosine	12	11	10
Phenylalanine	9	9	8
Total	310	310	310

[a] Each value represents the average of duplicate analyses. Calculations based on β chain molecular weight of 40,000 and carbohydrate content of 15%.

6. Bindings studies. The effect on the binding ability of hp 1-1 of the various treatments employed during chain preparation is shown in Table II (a). The binding activity of the chains prepared by both procedures is shown in Table II (b). These tables show that extensive reduction destroyed all binding activity whereas the milder method of reduction allowed considerable binding activity to be retained.

7. Immunological studies. The anti hp 2-2 rabbit serum used for these studies did not show a spur between normal free hp 2-2 and hp 1-1 (Fig. 4a). The β_{2-2} and β_{1-1} chains obtained by the milder reductive method showed a strong line of precipitation at concentrations of 1 mg/ml and a reaction of identity with native hp 2-2 as well as hp 1-1.

The α_{2-2} or α_{1-1} chains obtained after milder reduction did not give a line of precipitation nor did the intrawell absorption technique show any other evidence of binding to antibody (not illustrated).

Immunological binding studies are shown in Fig. 4b. Intact hp 1-1, as well as isolated β chains obtained after milder reduction, all showed a spur between the free preparations and their complexes with hemoglobin. This indicates that these β chains were still capable of binding hemoglobin.

The extensively reduced material lost all ability to interact with antibody. As shown in Fig. 4c hp 1-1 and 2-2, fully reduced but unfractionated, as well as β_{2-2} chain, isolated after full reduction, could not precipitate antibody. Intrawell absorption studies showed that there was no nonprecipitating interaction between these preparations and the antiserum (not illustrated).

Discussion. The preparation of haptoglobin polypeptide chains is complicated by their structural diversity. On the one hand, all the evidence hitherto available indicates that the β chains of the three common types of haptoglobin are similar. On the other hand, the α_{2-2} chain is approximately twice as large as the α_{1-1} chain and tends to aggregate in neu-

TABLE II. (a) Effect of Various Treatments on Binding Ability of Haptoglobin 1-1.

Treatment	Mg hb bound/mg test material	Percentage of hb bound taking purified hp 1-1 as 100%
1. Purification of hp 1-1	0.66 (corresponds to 1 mole hb per mole hp)	100
2. Dialysis vs 5 *M* guanidine HCl, 0.05 *M* Tris, pH 7.75		
(a) for 2 days	0.44	66
(b) for 7 days	0.30	46
3. Reduction 0.02 *M* mercaptoethanol, alkylation 0.24 *M* iodoacetamide. No guanidine present	0.15	23
4. Reduction and alkylation as in 3 above, then dialysis vs 5*M* guanidine HCl, 0.05 *M* Tris, pH 7.75 for 2 days. Dialysis vs *N* saline for 3 days	0.12	18
5. Reduction 0.2 *M* mercaptoethanol and alkylation 0.4 *M* iodoacetamide in presence of 5 *M* guanidine HCl, 0.25 *M* Tris, pH 8.2. Dialysis vs *N* saline for 3 days	0	0

TABLE II. (b) Binding Ability of β Chains Prepared by Mild and Extensive Reduction.

Preparation	Chain	mg hb bound/ mg test material	Percentage of hb bound taking purified hp 1-1 as 100%
1. Reduction 0.2 *M* mercaptoethanol and alkylation 0.24 *M* iodoacetamide, both in absence guanidine. Then separation of chains on G-200 in 5 *M* guanidine HCl, 0.05 *M* Tris, pH 7.75 (2 days).	$\beta_{2\text{-}2}$	0.10	15
	$\alpha_{2\text{-}2}$	0	0
	$\beta_{1\text{-}1}$	0.23	35
	$\alpha_{1\text{-}1}$	0	0
2. Reduction 0.2 *M* mercaptoethanol and alkylation 0.4 *M* iodoacetamide, both in presence of 5 *M* guanidine HCl, 0.25 *M* Tris, pH 8.2. Then separation of chains on G-200 in 5 *M* guanidine HCl, 0.05 *M* Tris, pH 7.75 (2 days).	$\beta_{2\text{-}2}$	0	0
	$\alpha_{2\text{-}2}$	0	0
	$\beta_{1\text{-}1}$	0	0
	$\alpha_{1\text{-}1}$	0	0

tral solution (5) so that the separation of α_2 chains from β chains is more difficult than that of α_1 chains.

Connell and co-workers used sodium dodecyl sulfate to precipitate β chains differentially after reductive cleavage (5). This method of preparing haptoglobin polypeptide chains is convenient for the structural study of all types of α chain, but is especially unsuitable for the preparation of $\beta_{2\text{-}2}$ chains, since half of the $\alpha_{2\text{-}2}$ chain is co-precipitated with the $\beta_{2\text{-}2}$ chain.

Shim and co-workers used acidic 4 *M* urea solutions during gel filtration and obtained the full theoretical yield of $\beta_{2\text{-}2}$ in the case of hp 2-2 (17), but it is unlikely that any binding

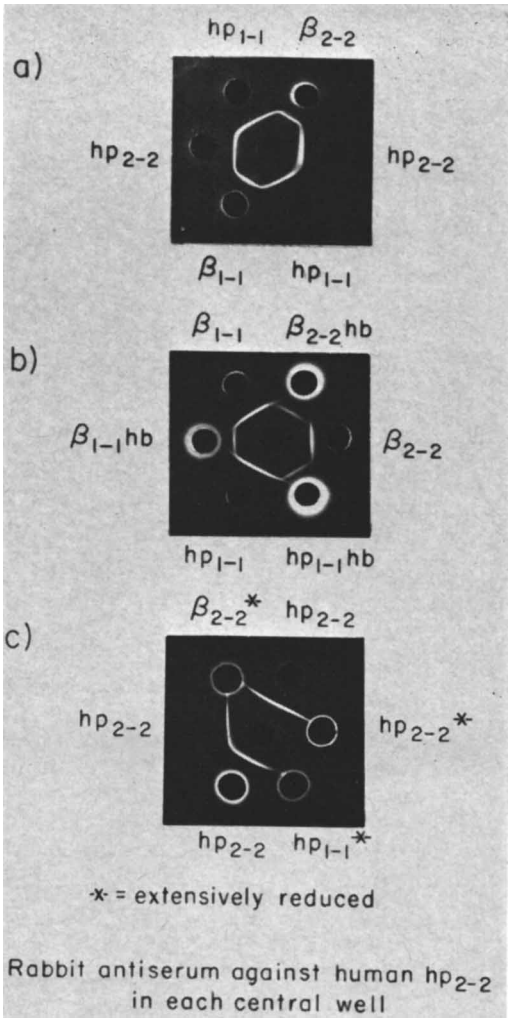

FIG. 4. Gel diffusion studies of haptoglobin and haptoglobin chains using a specific rabbit anti-hp 2-2 serum: (a) purified haptoglobins 1-1 and 2-2 compared with β chains isolated by the milder method of reduction; (b) the ability of less reduced β chains to bind hemoglobin; and (c) extensively reduced material compared with normal hp 2-2.

activity would have been present after these chains had been exposed to both acid and urea.

In the study described here we found that 5 *M* guanidine solutions used at neutral pH could achieve complete dissociation of all types of haptoglobin polypeptide chains. Moreover, when reduction and alkylation had been performed in the absence of guanidine the chains subsequently obtained with the use of guanidine as a dissociating medium could recover considerable hemoglobin binding activity when the guanidine was removed.

Guanidine is therefore a useful dissociating solvent for the preparation of haptoglobin polypeptide chains. Similar advantages have been found for the preparation of polypeptide chains of immunoglobulins (18).

When reduction was performed in the absence of guanidine, some disulfide bonds remained intact. It was possible, however, to separate pure β_{2-2} and α_{2-2} chains from undissociated material by means of gel filtration on Sephadex G-200 in guanidine. These β_{2-2} chains regained a substantial amount of binding activity after removal of the guanidine. This could be demonstrated spectrophotometrically by means of a peroxidase assay as well as immunologically. The β_{2-2} chains showed a reaction of identity with hp 1-1 and hp 2-2 when tested against a specific antihaptoglobin serum. The ability of these β_{2-2} chains to bind hemoglobin was confirmed by the formation of a spur between the β_{2-2} chain and β_{2-2}-hemoglobin complex.

The α_{2-2} chains obtained after less extensive reduction did not reveal immunological activity. This could be attributed to the antiserum used rather than to damage to the chain, since the antiserum could not distinguish between native hp 2-2 and hp 1-1 and presumably did not contain antibodies directed against the α_{2-2} chain. The isolated α_{2-2} chains showed no binding activity in the peroxidase assay system either.

When hp 1-1 was less extensively reduced only 14% of α_{1-1} chain could be obtained relative to the amount of β_{1-1} chain, instead of the theoretical yield of 18%. This was due to a contaminant of the β_{1-1} chain fraction. The relatively higher binding activity of the β_{1-1} preparation when compared with that of β_{2-2} (Table II b) cannot be evaluated because of the presence of this contaminant. The α_{1-1} chains obtained by this method are electrophoretically pure, but, as in the case of α_{2-2}, showed no ability to bind hemoglobin or specific antibody.

These studies confirm our earlier findings which demonstrated the binding ability of isolated β chains but not α chains when these chains were prepared with 0.01 *N* propionic

acid as the dissociating medium and tested for binding ability by means of gel filtration (6). The demonstration that fully reduced material lost all binding ability accounts for the finding of Lisowska and Dobryszycka that β chains isolated after reduction in 8 *M* urea could not bind hemoglobin (19). Evidence has therefore been obtained by two different methods of chain fractionation that the β chain makes the major contribution to the haptoglobin binding site for hemoglobin.

The use of guanidine with Sephadex G-200 gel filtration has not hitherto been explored as a method of estimating the size of proteins. By its effect on molecular conformation guanidine could eliminate irregularities found on gel filtration and due to variation in shape among different proteins (20). The known molecular weights for $\alpha_{2\text{-}2}$, $\alpha_{1\text{-}1}$ and Gc and the presumptive molecular weight of β chains show that their chromatographic behavior in this system agrees well with expected behavior. Although both the Sephadex G-200 gel particles and the proteins "swell" considerably in guanidine, the effect on proteins must be greater since the exclusion volume has diminished from a molecular weight range of about 200,000 to about 64,000. This system could be useful for estimating the size of subunits associated by strong noncovalent interactions.

All the evidence available from studies of size, electrophoretic behavior, amino acid analysis and peptide mapping (to be reported elsewhere) indicate the identity of β chains prepared from the 3 common genetic types of haptoglobin. This favors the hypothesis that the genetic heterogeneity of common haptoglobins is entirely due to the α chain locus (15). If there is no difference between $\beta_{1\text{-}1}$ and $\beta_{2\text{-}2}$, the polymeric system of hp 2-2 should be due to the properties of the $\alpha_{2\text{-}2}$ chain, as other authors have already suggested (5).

Very little is as yet known of the mechanism by which haptoglobin interacts with hemoglobin. Further studies of the β chains of haptoglobin should be of considerable interest in the study of this problem.

Summary. An improved method is described to prepare haptoglobin polypeptide chains in high yield and purity. Reduction and alkylation in the presence or absence of guanidine were followed in both cases by fractionation on Sephadex G-200 in guanidine solutions at neutral pH. Extensive reduction in the presence of guanidine resulted in denatured chains suitable for structural study whereas milder reduction in the absence of guanidine yielded β chains capable of binding hemoglobin. Some physical, chemical, binding, and immunological studies on the isolated chains are described. The β chains of the common types of haptoglobin showed similarity in all these properties.

1. Smithies, O., Biochem. J. **61**, 629 (1955).
2. Connell, G. E., Dixon, G. H., and Smithies, O., Nature **193**, 505 (1962).
3. Shim, B-S and Bearn, A. G., J. Exptl. Med. **120**, 611 (1964).
4. Connell, G. E., Smithies, O., and Dixon, G. H., J. Mol. Biol **21**, 213 (1966).
5. Connell, G. E., Smithies, O., and Dixon, G. H., J. Mol. Biol. **21**, 225 (1966).
6. Gordon, S. and Bearn, A. G., Proc. Soc. Exptl. Biol. Med. **121**, 846 (1966).
7. Muller-Eberhard, U., Scand. J. Clin. Lab. Invest. **12**, 33 (1960).
8. Herman-Boussier, G., Moretti, J., and Jayle, M-F., Bull. Soc. Chim. Biol. **42**, 817 (1960).
9. Kielley, W. W. and Harrington, W. F., Biochim. Biophys. Acta **41**, 401 (1960).
10. Edelman, G. M and Poulik, M. D., J. Exptl. Med. **113**, 861 (1961).
11. Bowman, B. H. and Barnet, D. R., Biochem. Biophys. Res. Commun. **26**, 466 (1967).
12. Muller, C. J., in "Molecular Evolution," p.15. Van Gorcum, Assen., 1961.
13. Tarukoski, P. H., Scand. J. Clin. Lab. Invest. **18**, 80 (1966).
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).
15. Berg, K., Acta Pathol. Microbiol. Scand. **59**, 369 (1963).
16. Scheidegger, J. J., Intern. Arch. Allergy Appl. Immunol. **7**, 103 (1955).
17. Shim, B-S, Lee, T-H, and Kang, Y-S, Nature **207**, 1264 (1965).
18. Lamm, M. E., Nussenzweig, V., and Benacerraf, B., Immunology **10**, 309 (1966).
19. Lisowska, E. and Dobryszycka, W., Biochim. Biophys. Acta **133**, 338 (1967).
20. Andrews, P., Biochem. J. **91**, 222 (1964).

Received June 6, 1967. P.S.E.B.M., 1968, Vol. 127.