[12] Bremer, H., and M. W. Konrad, these PROCEEDINGS, **51**, 801 (1964).
[13] Hurwitz, J., J. J. Furth, M. Anders, and A. Evans, *J. Biol. Chem.*, **237**, 3752 (1962).
[14] Berg, P., R. D. Kornberg, H. Fancher, and M. Dieckmann, *Biochem. Biophys. Res. Commun.*, **18**, 932 (1965).
[15] Marmur, J., and C. M. Greenspan, *Science*, **142**, 387 (1963).
[16] Tocchini-Valentini, G. P., M. Stodolsky, M. Sarnat, A. Aurisicchio, F. Graziosi, S. B. Weiss, and E. P. Geiduschek, these PROCEEDINGS, **50**, 935 (1963).
[17] Colvill, A. J. E., L. C. Kanner, G. P. Tocchini-Valentini, M. T. Sarnat, and E. P. Geiduschek, these PROCEEDINGS, **53**, 1140 (1965).
[18] Fowler, A. V., J. Marmur, and J. Hurwitz, unpublished observations.
[19] Cairns, J., *J. Mol. Biol.*, **6**, 208 (1963).
[20] Ralph, R. K., R. J. Young, and H. G. Khorana, *J. Am. Chem. Soc.*, **85**, 2002 (1963).
[21] Bell, D., R. V. Tomlinson, and G. M. Tener, *Biochem. Biophys. Res. Commun.*, **10**, 304 (1963).

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*REDUCTIVE DISSOCIATION OF CHICKEN γG IMMUNOGLOBULIN IN NEUTRAL SOLVENTS WITHOUT A DISPERSING AGENT**

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The γG immunoglobulins (γG) of different animal species consist of two types of polypeptide chains: the light (L) chains (K or L chains) with a molecular weight of 20,000, and the heavy (H) chains (γ chains) with a molecular weight of about 55,000.[1-5] The chains are held together by disulfide linkages; however, reduction of the disulfide bonds and alkylation of the released −SH groups has not been reported to lead to chain separation unless followed by treatment with urea,[2] detergent,[6, 7] or weak acid.[5] Thus, either reduced human or rabbit γG in neutral buffer retains most of its antibody-combining activity and has approximately the same sedimentation coefficient as unreduced γG.[2, 8] In contrast, chicken γG antibody loses considerable activity following reduction with 2-mercaptoethanol (ME).[9] The results presented here show that, unlike rabbit γG, fractionation of reduced alkylated chicken γG in neutral or alkaline buffers yields free H and L chains.

Materials and Methods.—Preparation of γG: The globulins were precipitated with Na_2SO_4[9] from pooled sera of adult White Leghorn hens, and the γG was isolated by gel filtration on Sephadex G-200 (Pharmacia, Upsala, Sweden) in borate-buffered saline, pH 8.2, $\tau/2 = 0.16$ (borate). The $S_{20,w}$ values of γG in these preparations ranged from 6.9 to 7.1*S*. Traces of β-globulin, detected by immunoelectrophoresis (IE), was eliminated in some preparations by chromatography through DEAE-cellulose in 0.1 *M* sodium phosphate buffer, pH 6.4.[9] Sodium sulfate-precipitated rabbit γG, purified by DEAE-cellulose chromatography,[10] had a sedimentation coefficient of 6.4*S* and consisted only of γG by immunologic criteria.

Protein concentrations were determined either from measurements of absorbance at 280 mμ or by the Folin reaction as described previously.[11]

Reduction and fractionation: One to 3% solutions of γG preparations in 0.55 *M* Tris-HCl buffer, pH 8.2, were reduced with various concentrations of ME at room temperature for 1 hr. In some experiments, dithiothreitol[12] (DTT) (Calbiochem) was used as the reducing agent. A 1 *M* solution of recrystallized iodoacetamide in the same buffer was added after reduction to a molar concentration 1.5 times that of the reducing agent, and the pH was maintained at 8.0 by

addition of 1 N NaOH. After 1 hr alkylation at room temperature, the reduced preparations were dialyzed against water. Preparations fractionated according to the procedure of Fleischman *et al.*[5] were subsequently dialyzed against 1 M propionic acid, pH 2.4 at 4°C, and those preparations fractionated under alkaline conditions were dialyzed against borate at 4°C. Separation of the chains was accomplished in either Sephadex G-75 or G-200 columns (4.5 × 50-cm), each equilibrated in 1 M propionic acid at room temperature. The same size column of Sephadex G-200 equilibrated in borate was used to fractionate alkaline solutions of reduced alkylated γG. Fractions were concentrated either by dialysis against water followed by lyophilization or by ultrafiltration.

Starch gel electrophoresis in 8 M urea was performed in 0.05 M formic acid at a constant current of 45 ma for 20 hr at room temperature.[2]

Papain digestion: Chicken γG was digested with papain as described previously.[11] The Fc fragment was prepared by washing the water-insoluble fraction of digested γG with cold water and dissolving in phosphate-buffered saline, pH 7.2. The water-soluble fraction was dialyzed against 0.005 M sodium phosphate buffer, pH 8.0, and the Fab fragment was isolated by passage through DEAE-cellulose.[11]

Ultracentrifugation: Sedimentation velocities ($S_{20,w}$) were determined as described previously.[11] The per cent relative composition of sedimenting proteins was determined by area measurements. Preparations run in 1 M propionic acid, pH 2.4, were corrected to $S_{20,w}$ using the factor 1.2.[6]

Immunodiffusion: The gel diffusion method of Ouchterlony[13] and the microimmunoelectrophoresis method of Scheidegger[14] were employed. Antisera to γG, H and L chains, and to Fab and Fc fragments, were produced by a single injection into rabbit footpads of 2–4 mg of each preparation in complete Freund's adjuvant. Antisera were made specific by absorption procedures. Antisera to whole-chicken globulin were prepared by giving rabbits several intramuscular injections of salt-precipitated globulins in complete Freund's adjuvant.

Results.—Reduced and acidified γG: Reduced alkylated γG was eluted from Sephadex G-75 in 1 M propionic acid in two fractions in a manner similar to that described by Fleischman *et al.*[5] From three different fractionations, the first and second fractions represented averages of 77 and 23 per cent of the total proteins eluted. Better resolution of the fractions resulted from filtration in Sephadex G-200, and three peaks were eluted (Fig. 1*C*). Sephadex G-200 was used exclusively in the remainder of this study. In the ultracentrifuge, the first fraction contained rapidly sedimenting aggregates. The second peak eluted from the column later than did unreduced γG (Fig. 1*A*), and consisted of protein characteristic of H chains. A concentrated pool of the second peak, refiltered through Sephadex, eluted as 88 per cent H chains and 12 per cent smaller material; the latter was presumed to be contaminating L chains. The recycled H chains had a sedimentation coefficient of 2.3S (Fig. 2*C* and Table 1). On dialysis of H chain preparations against water, a large amount of precipitate formed. In the soluble fraction at pH 8.2, protein sedimented as a single 5.8S component (Table 1) and was presumed to be dimers[15] of H chains. Approximately 24 per cent of the protein eluted from Sephadex as L chains (L chains in refiltered second peak plus third peak shown in Fig. 1*C*). The sedimentation coefficients of the L chains at pH 2.4 and pH 8.2 were 1.8S and 3.6S, respectively (Figs. 2*B* and *D*).

The aggregates in the first Sephadex fraction failed to precipitate in agar with antisera against γG, L chains, H chains, Fab, and Fc. The anti-H chain serum was obtained from rabbits which had been injected with a Sephadex G-75 H chain fraction consisting of aggregates and of 2.3S material. In IE, the second Sephadex fraction (H chains) formed a weak precipitin arc around the antigen well with anti-γG antisera. In Ouchterlony plates, H chains, which had been dialyzed against borate, produced a precipitin line after 4–5 days with anti-γG, anti-H, anti-Fc, and

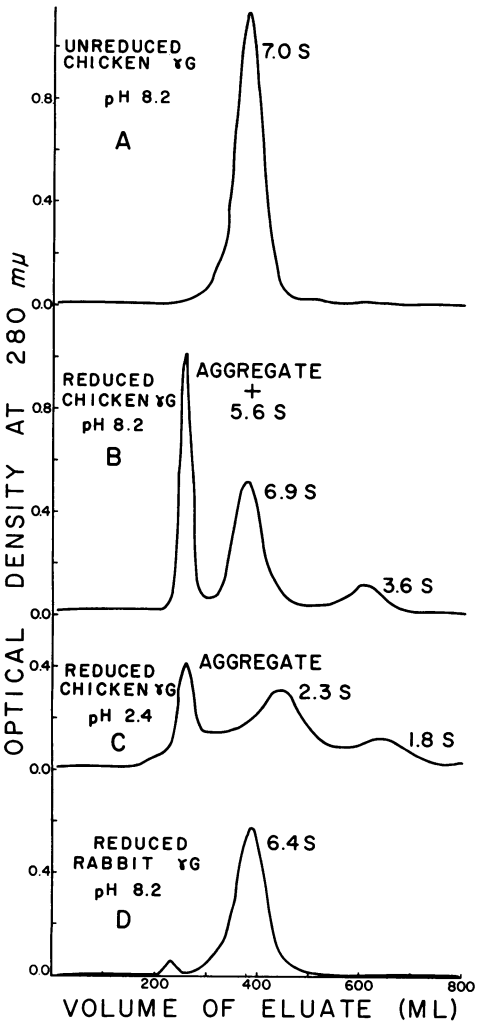

FIG. 1.—Gel filtration of unreduced γG, and γG reduced with 0.2 *M* ME, on Sephadex G-200 in borate (*A*), (*B*), and (*D*); and in 1 *M* propionic (*C*). Eighty mg γG used in (*A*), (*B*), and (*C*); 40 mg in (*D*).

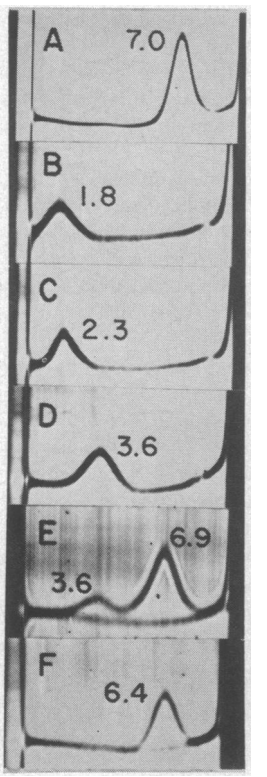

FIG. 2.—Sedimentation patterns after 78 min at 59,780 rpm. (*A*) Unreduced chicken γG; (*B*), (*C*), and (*D*), chicken L, H, and L chains, respectively; (*E*) and (*F*), chicken and rabbit γG, respectively, reduced with 0.2 *M* ME. Solvent: borate, pH 8.2 (*B* and *C*, 1 *M* propionic, pH 2.4). The numerals are $S_{20,w}$ values.

no line with anti-Fab. Purified H chains stored in propionic acid for 3 days did not precipitate with any antiserum. The L chains, recovered from Sephadex, reacted with anti-γG, anti-L, and anti-Fab sera to form a single heavy, long arc around the antigen well in IE (Fig. 4*B*). Anti-Fc and anti-H sera failed to precipitate L chains.

In urea starch gel electrophoresis, reduced γG appeared similar to that described by Edelman.[16] Sephadex-recycled H chains migrated as a single distinct band with a mobility approximately the same as that of human and rabbit H chains. Purified L chains were more diffuse and formed two broad bands: one slightly faster, but close to the H chain; and a second diffuse band with a faster mobility.

Thus, the properties of reduced and acidified chicken γG generally resembled

TABLE 1

SEDIMENTATION COEFFICIENTS OF γG CHAINS

Fraction	pH	Protein conc. (mg/ml)	$S_{20,w}$
Chicken γG			
Unreduced	8.2	10.0	7.0
Reduced*	8.2	10.0	3.6, 6.9†
L chain‡	2.4	5.0	1.8
L chain‡	8.2	5.0	3.6
L chain§	8.2	5.0	3.6
H chain‡	2.4	5.0	2.3
H chain‡	8.2	3.0	5.8
Rabbit γG			
Unreduced	8.2	10.0	6.4
Reduced	8.2	8.5	6.4

* Chicken and rabbit γG reduced with 0.2 *M* ME, alkylated, and dialyzed against borate.
† $S_{20,w}$ of aggregates not calculated.
‡ γG reduced with 0.2 *M* ME, alkylated, and passed through Sephadex G-200 in 1 *M* propionic.
§ γG reduced with 0.2 *M* ME, alkylated, and passed through Sephadex G-200 in borate.

the properties of reduced γG of other animal species.[5] However, reduced alkylated chicken γG in either neutral or alkaline buffers formed a new precipitin arc, thus suggesting the presence of either some intact γG molecules with new electrophoretic mobilities or of dissociation products. The following experiments present evidence that free L chains were detected.

Sedimentation properties at pH 8.2 of reduced γG: Ultracentrifugal examination of γG, which had been reduced with 0.2 *M* ME, alkylated, and dialyzed against borate, revealed three sedimenting components: rapidly sedimenting aggregates (26*S* and above), and 3.6*S* and 6.9*S* peaks (Fig. 2*E* and Table 1). From area measurements of the aggregates made in a double-sector cell 2 min after reaching speed, the aggregates represented 38 per cent of the total proteins. Sixty-two min after reaching speed the 7*S* and 3.6*S* peaks were 46 and 15 per cent of the total areas. Therefore, approximately 54 per cent of γG was dissociated, and, assuming the aggregates to be H chains, the relative yields from dissociated γG were 71 per cent H chains and 29 per cent 3.6*S* component. This agrees closely with the ratio of H to L chains obtained by reduction and acidification.

Preparations treated with 0.05 and 0.1 *M* ME had little or none of the 3.6*S* component (Table 2). A large amount of precipitate formed after alkylation when ME concentrations above 0.2 *M* were used; however, the per cent loss of protein by precipitation was less when lower concentrations of protein were reduced. Little precipitation occurred when solutions of 0.8 per cent γG were reduced with 0.5 *M* ME, and the soluble supernatant fraction of such a preparation contained only 3.6*S* material (Table 2).

TABLE 2

PER CENT DISSOCIATION PRODUCTS ($S_{20,w}$) OF REDUCED γG AT pH 8.2

Molarity of ME*	Relative Per Cent of: 3.6*S*	7.0*S*	Aggregates
0	0	100	0
0.05	0	100	0
0.10	3	97	NM
0.20	15	46	38
0.50†	100	0	NM

* γG concentration: 25 mg/ml.
† γG concentration: 8 mg/ml. NM = area not measured.

Gel filtration at pH 8.2 of reduced γG: Preparations reduced with 0.2 *M* ME, alkylated, and passed through Sephadex G-200 in borate, eluted in three fractions (Fig. 1*B*). The yields of the fractions agreed very closely with the ultracentrifugal data. The first peak, which consisted of rapidly sedimenting material and a small amount of 5.6*S* protein (probably dimers of H chains), represented 39 per cent of the total protein eluted. The second peak contained only undissociated γG with a sedimentation coefficient of 6.9*S* (48% of the total protein eluted); however, the second peak of one preparation, concentrated by lyophilization, had 3.6*S* material, and a 7*S* peak with a shoulder on the trailing side. The third Sephadex fraction (Fig. 1*B*) contained only the 3.6*S* component (Fig. 2*D*) and represented 13 per cent of the total protein. Alkylated unreduced γG (Fig. 1*A*) eluted from Sephadex in the same fraction as the second fraction of reduced γG at pH 8.2 (Fig. 1*B*), but both preparations eluted earlier than did the H chains produced by acidification (Fig. 1*C*). The L chains eluted from the acid column later than did L chains eluted from the alkaline column (Fig. 1). This was expected since the sedimentation velocities of the L chains at pH 2.4 and at 8.2 were 1.8 and 3.6, respectively.

Examination was made of the aggregates in preparations which had been reduced with high concentrations of ME, and which were eluted in the first Sephadex fraction. A solution containing 140 mg of γG was reduced with 0.5 *M* ME, and the precipitate (55 mg) which formed was dissolved in 8 *M* urea in 1 *M* propionic acid and passed through Sephadex G-200 equilibrated in the same reagent. Approximately 90 per cent of the protein eluted as free or aggregated H chains and 10 per cent as L chains. In urea starch gel electrophoresis this preparation contained only H chains.

Rabbit γG, reduced with 0.2 *M* ME and alkylated, eluted from Sephadex G-200 at pH 8.2 as 6.4*S* globulin (Fig. 1*D*), and no dissociation products were detected in the ultracentrifuge (Fig. 2*F*).

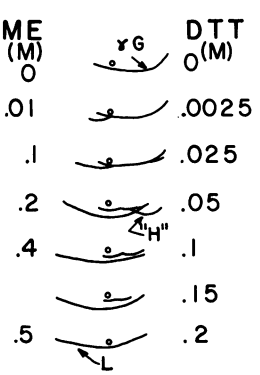

FIG. 3.—IE of γG reduced with increasing amounts of ME and of DTT. Rabbit anti-chicken globulin was added to the troughs.

Immunoelectrophoretic properties of reduced γG at pH 8.2: The 3.6*S* component, obtained from the third Sephadex peak (Fig. 1*B*), gave an arc in IE identical with that of the L chains produced by reduction and acidification (Fig. 4*B*). The minimal amounts of ME and DTT needed to release L chains in alkaline buffer was determined by IE employing anti-γG serum. The results are shown in Figure 3. As the concentrations of ME and DTT were increased, the L chain arc increased progressively. A small amount of L chain arc, which formed a line of partial identity with γG, appeared in preparations reduced with 0.05 *M* ME. Reduction with 0.4–0.5 *M* ME yielded only L chain precipitin arcs. A faint arc at the extreme cathodal end appeared when 0.1 or 0.2 *M* ME were used for reduction (Figs. 3 and 4*B*), formed a line of partial identity with that of γG, and crossed the L chain line. This component could be detected only with antisera for whole globulin and for Fc fragments. Although this band has not yet been detected with anti-H serum, the data suggest that this component may be H chains and that only a part of the native H chain determinants were available for reaction.

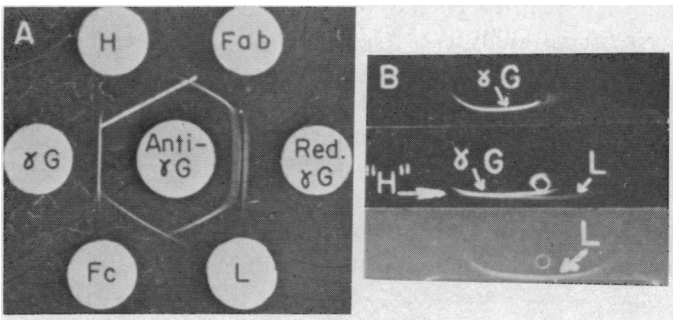

FIG. 4.—(*A*) Double diffusion of γG, γG reduced with 0.2 *M* ME (Red. γG), H and L chains prepared by gel filtration at pH 2.4 and adjusted to pH 8.2, and Fc and Fab fragments. H chain line faint; line drawn. (*B*) (from top to bottom) IE of: γG, Red. γG, and L chains prepared as described above. Rabbit antichicken globulin was used in (*A*) and (*B*).

On a molar basis, DTT was a more active reducing agent than ME. The L chain arc was detected when γG was reduced with 0.0025 *M* DTT, and no undissociated γG was detected when γG was reduced with 0.25 *M* DTT (Fig. 3). The "H chain" arc was detected with 0.05 *M* DTT and disappeared at higher concentrations of DTT. Use of DTT was discontinued because it induced severe headaches in the investigators.

Antigenic studies: The L chains prepared in acid formed a line of identity with the inside band (L chains) of preparations reduced and partially dissociated in alkaline buffer (Fig. 4*A*) and with the 3.6*S* component obtained by Sephadex fractionation at pH 8.2. Gel diffusion has proved to be the most sensitive method for detection of free L chains in reduced alkylated preparations maintained in neutral or alkaline buffers. As shown in Figure 4*A*, the L chains, prepared either at pH 2.4 or pH 8.2, were antigenically unrelated to the H chains and to the Fc fragment; and, as expected, Fab and Fc fragments were antigenically distinct. The Fab fragment appears to be distinct from the H chains, thus implying nonidentity between the H chains produced in acid and the H chain determinants in the Fd fragment. As expected, undissociated γG spurred with Fab fragments and the L chains contained some, but not all, of the antigenic determinants of the Fab fragments.

Discussion.—Similar to reduced γG of other species,[5] reduced alkylated chicken γG elutes as two antigenically distinct fractions from Sephadex G-75 in 1 *M* propionic acid with relative yields of 76 and 24 per cent, respectively. At pH 2.4 they had sedimentation coefficients of 2.3*S* and 1.8*S*, respectively. These coefficients agree with the values reported for isolated rabbit H and L chains.[5] In neutral buffer, the chains form dimers,[15] and the sedimentation coefficients of H and L chain dimers of rabbit γG are 6.1*S* and 3.2*S*, respectively.[6] Chicken H and L chains had values of 5.8*S* and 3.6*S*, respectively, at pH 7.2 or 8.2, suggesting dimerization.

Edelman and Poulik[2] reported no dissociation products from reduced alkylated human and rabbbit γG maintained in neutral buffers without a dispersing agent. However, chicken γG reduced with 0.2 *M* ME dissociated in borate to yield rapidly sedimenting aggregates, 7*S* and 3.6*S* materials; approximately half of the native molecule dissociated. Rosenquist and Gilden[17] also noted that chicken 7*S* globulin, reduced with 0.1 *M* ME at pH 7.2, had a detectable amount of a 3.6*S* component.

Aggregated H chains, intact 7*S* globulin, and L chains were separated in

Sephadex G-200 at pH 8.2. The relative yield of each species agreed closely with values obtained by ultracentrifugal analysis. Assuming that the first Sephadex peak contained aggregated H chains, and excluding undissociated γG from calculations, the yields of H and L chains were 76 and 24 per cent, respectively. The same relative yields were obtained by fractionation in Sephadex G-75 at pH 2.4. In double diffusion tests the 3.5*S* fraction, produced at pH 8.2, formed a line of complete identity with L chains, which had been prepared by the method of Fleischman *et al.*,[5] and failed to be precipitated by anti-H and anti-Fc sera. In IE, the 3.5*S* component formed an arc in the same position as those of rabbit[18] and of chicken L chains.

Schur and Christian[19] reported that reduction of rabbit γG with 0.85 *M* ME at pH 6.9 resulted in the formation of 25 per cent 3.5*S* material, and that sheep γG reduced with 0.075 *M* ME resulted in 10 per cent 3.8*S* protein. It would be interesting to know whether their 3.8*S* components were L chain dimers, such as we found in the present study.

Grossberg *et al.*[20] reported that Fab fragment of rabbit γG did not dissociate in neutral buffers after reduction with 0.35 *M* ME. The noncovalent bonds binding the reduced chicken γG polypeptide chains are probably weaker than the noncovalent bonds of rabbit γG. Apparently, the bonds holding the L and H chains together are easily broken in neutral or alkaline buffers. Reduction with 0.1 *M* ME yielded 3 per cent 3.6*S* material, and reduction with 0.2 *M* ME produced 15 per cent 3.6*S* material. Since a concentration of 0.1 *M* ME is sufficient to break interchain disulfide bonds, as evidenced by complete fragmentation of γG in acid, higher concentrations of ME may be breaking intrachain disulfide bonds, thereby altering the tertiary structure of the molecule and weakening the noncovalent bonds. Intermolecular forces binding the reduced polypeptide chains are dependent, in part, on primary structure, and differences in the amino acid composition between chicken γG and γG from other species would account for these findings. In preliminary experiments, serine, glycine, and alanine were present in relatively higher concentrations, and glutamic acid and tyrosine in relatively lower concentrations, in chicken γG[11] than in γG of other species.[21]

Detection of chicken H chains by immunologic methods has been difficult. Detection may depend on the concentration of reducing agent, on the pH used for fractionation, and on the immunogenicity of H chains. Although anti-H serum forms a heavy precipitin line with γG, it precipitates H chain preparations weakly; and, in IE, H chains made from reduced acidified γG form only a weak arc around the antigen well. Furthermore, L chains produced heavier precipitin bands than did the H chains with anti-γG serum, even though H chain preparations contained ten times more protein. In addition to the L chain and to undissociated γG bands, a third component was detected in preparations which had been reduced with 0.1 and 0.2 *M* ME and kept at pH 7.2–8.2. In IE, this component migrated toward the extreme cathodal end and was detected only with antiwhole globulin and anti-Fc sera, and not with anti-Fab, anti-L, or anti-H sera. The anti-H serum, made by immunizing with H chains prepared in acid, failed to detect this component in IE. In addition, this arc was detected only when γG was reduced with certain concentrations of ME (0.1–0.2 *M*). At low concentrations of reducing agent, a sufficient concentration of H chains may not be present, and at higher concentra-

tions of ME, additional disulfide reduction (possibly intrachain) may have altered the Fc antigenic determinants. We suggest, therefore, that the antigenic determinants represented on the Fc fragment may have been altered both by reduction and by acid treatment. These observations also may be explained on the basis that antibody produced to acid-dissociated chicken H chains may be directed to different antigenic determinants than is antibody specific for intact γG or for papain-produced subunits. Indeed, H chain preparations which fail to precipitate with antisera after a few days' storage in acid may have undergone further unfolding.

The data indicate that the structure of chicken γG is consistent with the 4-chain structure proposed by Porter[22] involving one pair of L chains and one pair of H chains. The relative yields of dissociated chains of chicken γG, and the antigenic relationships between isolated chains and enzymatically produced fragments fit the 4-chain theory. The Fab fragment was composed of L chain determinants and a portion of an H chain, and Fc fragment contained only part of the antigenic determinants of the H chain. The Fc fragment formed a line of partial identity with intact γG with anti-H serum, and the spur formed was small and markedly deviated. Although there is a high degree of relationship between Fc and γG, the antigenic determinants of Fd may have been detected with the anti-H serum. In this connection, anti-H serum failed to precipitate Fab; possibly the H chain determinants in Fab fragment are destroyed by papain digestion. Substantiating this hypothesis, anti-Fab failed to precipitate the H chain preparations, but it should be pointed out that chicken H chain preparations were weak precipitating antigens. Clarification of this point awaits further work.

Dissociation at neutral pH of reduced alkylated and nonalkylated chicken γG explains, in part, why chicken γG antibody loses some of its various antibody activities.[9, 17, 23, 24]

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[1] *Bull. World Health Organ.*, **30**, 447 (1964).

[2] Edelman, G. M., and M. D. Poulik, *J. Exptl. Med.*, **113**, 861 (1961).

[3] Franek, F., *Biochem. Biophys. Res. Commun.*, **4**, 28 (1961).

[4] Ramel, A., E. Stellwagen, and H. K. Schachman, *Federation Proc.*, **20**, 387 (1961).

[5] Fleischman, J. B., R. H. Pain, and R. R. Porter, *Arch. Biochem. Biophys.*, Suppl. 1, 174 (1962).

[6] Jaquet, H., B. Bloom, and J. J. Cebra, *J. Immunol.*, **92**, 991 (1964).

[7] Utsumi, S., and F. Karush, *Biochemistry*, **3**, 1329 (1964).

[8] Deutsch, H. F., and J. I. Morton, *Science*, **125**, 600 (1957).

[9] Benedict, A. A., R. J. Brown, and R. T. Hersh, *J. Immunol.*, **90**, 399 (1963).

[10] Peterson, E. A., and H. A. Sober, *J. Am. Chem. Soc.*, **78**, 751 (1956).

[11] Dreesman, G., and A. A. Benedict, *J. Immunol.*, in press.

[12] Cleland, W. W., *Biochemistry*, **3**, 480 (1964).

[13] Ouchterlony, O., *Acta Pathol. Microbiol. Scand.*, **32**, 231 (1953).

[14] Scheidegger, J. J., *Intern. Arch. Allergy Appl. Immunol.* **7**, 103 (1955).

[15] Fleischman, J. B., R. R. Porter, and E. M. Press, *Biochem. J.*, **88**, 220 (1963).

[16] Edelman, G. M., in *Immunopathology*, Third International Symposium, La Jolla, 1963, ed. P. Grabar and P. A. Miescher (Basel: Schwabe and Co., 1963), p. 57.
[17] Rosenquist, G. L., and R. V. Gilden, *Biochim. Biophys. Acta*, **78**, 543 (1963).
[18] Roholt, O., K. Onoue, and D. Pressman, these PROCEEDINGS, **51**, 173 (1964).
[19] Schur, P. H., and G. D. Christian, *J. Exptl. Med.*, **120**, 531 (1964).
[20] Grossberg, A. L., P. Stelos, and D. Pressman, these PROCEEDINGS, **48**, 1203 (1962).
[21] Cohen, S., and R. R. Porter, in *Advances in Immunology*, ed. F. J. Dixon, Jr., and J. H. Humprey (New York: Academic Press, 1964), vol. 4, p. 287.
[22] Porter, R. R., in *Basic Problems in Neoplastic Disease*, ed. A. Gelhorn and E. Hirschberg (New York: Columbia University Press, 1962), p. 177.
[23] Uhr, J. W., M. S. Finkelstein, and E. C. Franklin, *Proc. Soc. Exptl. Biol. Med.*, **111**, 13 (1962).
[24] Dreesman, G., C. Larson, R. M. Pinckard, R. M. Groyon, and A. A. Benedict, *Proc. Soc. Exptl. Biol. Med.*, **118**, 292 (1965).

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*ENZYMATIC SYNTHESIS OF DEOXYRIBONUCLEOTIDES, VIII. THE EFFECTS OF ATP AND dATP IN THE CDP REDUCTASE SYSTEM FROM E. COLI**

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The enzymatic formation of deoxyCDP from CDP in *Escherichia coli* B requires the participation of four different protein fractions.[1-3] Two of these (thioredoxin[2] and thioredoxin reductase[3]) have been obtained in an essentially pure form while the remaining two (enzymes B1 and B2) have hereto been available only in a relatively crude state.[1] Our present understanding of the interplay of these fractions in the formation of dCDP is summarized in Figure 1.

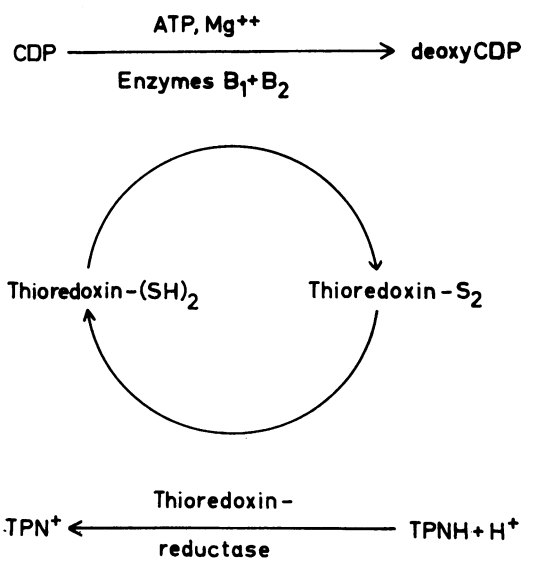

FIG. 1.—The CDP reductase system from *E. coli* B.