The use of ^{14}C measurements to follow the migration of amino acids assumes that no alteration occurs during passage through the cells. Ehrlich cells concentrate many amino acids rapidly and extensively, and only a few, *e.g.*, leucine, glutamine, and arginine are rapidly metabolized (Quastel, 1965). AIB is an unnatural amino acid and is not metabolized (Noall *et al.*, 1957).

Christensen *et al.* (1952b,c) associated the accumulation of amino acids with the loss of cell potassium. For optimal transport of amino acids into Ehrlich ascites cells, K^+ is required in the extracellular fluid (Christensen *et al.*, 1952a). On the other hand, Hempling and Hare (1961) suggest that amino acids (such as glycine) facilitate potassium transport rather than the reverse. Possibly different amino acids affect potassium transport in different ways. At present, not enough information is at hand to speculate on what happens to the individual rates 1–4. The system here described offers the possibility of studying these different rates by including measurements of the intracellular compartment C. The clarification of the individual rates will lead to a clearer picture of the function of the Ehrlich ascites cell membrane in the transport and exchange diffusion of amino acids under various conditions.

Acknowledgment

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Comparison of Guinea Pig γ_1- and γ_2-Immunoglobulins by Peptide Mapping*

Michael E. Lamm,† Barbara Lisowska-Bernstein, and Victor Nussenzweig

ABSTRACT: To enable comparison of two classes of immunoglobulins from the same animal species γ_1- and γ_2-globulins were obtained from guinea pig anti-2,4-dinitrophenyl antibodies. The fragments produced by the action of papain and pepsin, and the H and L chains resulting from extensive reduction were examined by peptide mapping after digestion with trypsin. These two classes of immunoglobulins contain similar Fab and $F(ab')_2$ fragments, identical L chains, half-similar H chains, and dissimilar Fc fragments. It is concluded that their Fd fragments are closely related and that class differences are localized largely to the Fc portions of the H chains. The Fc portions do, however, contain a number of similar peptides.

Guinea pigs offer certain advantages for comparing different classes of immunoglobulins within a single animal species. γ_1- and γ_2-globulin antibodies can be obtained in reasonable yields and relative purity (Benacerraf *et al.*, 1963), and for structural studies it is fortunate that both classes can be successfully subjected to standard procedures for preparing fragments by proteolysis and polypeptide chains by reduction and gel filtration. The fact that the Fc fragments of γ_1- and γ_2-globulins mediate distinct biological phenomena (Ovary *et al.*, 1963; Bloch *et al.*, 1963; Berken and Benacerraf, 1966) is of additional interest.

It is known that individual classes of immunoglobulins within a given animal species differ with regard to their Fc fragments but share common light poly-

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peptide chains (Cohen and Porter, 1964). There is also evidence that the Fd fragments of different classes may contain similar structural features. In rabbits the allotypes controlled by the a locus are present in IgG,[1] IgM, and IgA (Todd, 1963; Stemke and Fisher, 1965; Feinstein, 1963; Lichter, 1967; Sell, 1967). The Fd fragments of guinea pig γ_1- and γ_2-globulins contain similar antigenic determinants (Nussenzweig and Benacerraf, 1966). The Fd fragments of horse IgG and IgG (T) globulins, which are thought to be members of the same immunoglobulin class, are also similar antigenically (Weir and Porter, 1966; Weir *et al.*, 1966). Finally, certain human IgG, IgM, and IgA myeloma proteins contain common antigenic determinants which are not present on L chains or Fc fragments and are thought to be on the Fd fragments or related to conformational determinants (Kunkel *et al.*, 1966; Harboe and Deverill, 1966; Seligmann *et al.*, 1966).

In view of the foregoing we decided to study guinea pig γ_1- and γ_2-globulins by peptide mapping in an effort to learn how different their Fc fragments are and how similar their Fd fragments might be.

Materials and Methods

Preparation of γ_1- and γ_2-Globulins. In order to obtain sufficient amounts of γ_1-globulin immunization was necessary since normal guinea pig serum contains little γ_1-globulin. Hartley guinea pigs were injected with either highly or lightly conjugated DNP-BGG in complete Freund's adjuvant, initial dose (1 mg) in the footpads and two booster doses (0.4 mg each, no adjuvant) intradermally. The animals were bled 1 month after the initial dose. The highly conjugated preparations contained 61 moles of hapten/mole of protein and resulted in the production of predominantly γ_2 antibodies. The lightly conjugated preparations, containing 15 moles of hapten/mole, stimulated the production of γ_1- and γ_2-globulins in more nearly equal amounts. Anti-2,4-dinitrophenyl (anti-DNP) antibodies were recovered from immune sera according to the method of Farah *et al.* (1960) as described previously (Lamm *et al.*, 1966). The antibodies were separated into γ_1 and γ_2 fractions by chromatography on DEAE-cellulose (Oettgen *et al.*, 1965). The purity of the final preparations was determined by specific quantitative precipitation in a liquid medium of ^{131}I-labeled aliquots by rabbit antisera specific for γ_1- or γ_2-globulin in the zone of antibody excess (Nussenzweig *et al.*, 1966). Three preparations of γ_1 antibodies varied from 88 to 94% pure,[2] and two preparations of γ_2 antibodies were 94 and 100% pure. The amounts of contaminating γ_2-globulin in the γ_1 preparations did not complicate the interpretation of the peptide maps since spots peculiar to γ_2-globulins were never observed in γ_1 maps, therefore being present in amounts below the limits of detection by this technique. Normal γ_2-globulin was obtained by reacting fraction II proteins (Pentex, Inc.) batchwise with DEAE-cellulose in 0.01 M sodium phosphate (pH 8.0).

Preparation and Isolation of Fragments Obtained by Proteolysis. F(ab')$_2$ fragments were prepared by peptic (Worthington Biochemical Corp.) digestion (Nisonoff, 1964). γ_1- and γ_2-globulins were digested at pH 3.7 and 4.5, respectively (digestion of γ_1-globulin at pH 4.5 was incomplete). Papain digestion (mercuripapain, Pierce Chemical Co.) was performed essentially as described by Porter (1959). After 4 hr the digest was dialyzed against phosphate buffer (pH 7.6) containing 10^{-4} M iodoacetamide.

Fab fragments were isolated by adding an excess of DNP-BGG to the digest for 1 hr at 37° and then precipitating the DNP-BGG together with bound Fab fragments by the addition of streptomycin to 35 mg/ml (Nussenzweig and Benacerraf, 1964). The precipitate was washed three times with PBS (pH 7.6) containing streptomycin and then eluted with 2,4-dinitrophenol (DNPOH) for 30 min at 37°, also in the presence of streptomycin. The supernatant was dialyzed against PBS, and the Fab fragments plus remaining DNPOH were passed through Dowex 1-X8 (200–400 mesh) in PBS to remove dissociable hapten.

Fc$_{\gamma_2}$ fragments were prepared from the supernatant remaining after the addition of DNP-BGG and streptomycin to the papain digest of γ_2-anti-DNP antibodies. Streptomycin was dialyzed from the supernatant, excess DNP-BGG was added, and the mixture was incubated at 37° for 30 min. Then the combination of DNP-BGG, possible remaining Fab$_{\gamma_2}$, and Fc$_{\gamma_2}$ was filtered through Sephadex G-100 or G-200 in 0.005 M sodium phosphate (pH 7.6) (Figure 1). The second elution peak contained Fc$_{\gamma_2}$ fragments. Fc$_{\gamma_1}$ fragments crystallized from the papain digest of γ_1-anti-DNP antibodies (Nussenzweig and Benacerraf, 1964) and were washed with cold buffer.

Fab and F(ab')$_2$ fragments from both γ_1- and γ_2-globulins as well as crystalline Fc$_{\gamma_1}$ fragments solubilized in detergent were shown to be pure by immunochemical criteria. The former did not precipitate with anti-Fc antisera, and the last did not precipitate with anti-F(ab')$_2$ and anti-Fc$_{\gamma_2}$ antisera. The preparation of Fc$_{\gamma_2}$ fragments was slightly contaminated with Fab fragments as demonstrated by immunoelectrophoresis, but again peptides characteristic of Fab-fragments were not observed in the maps of Fc$_{\gamma_2}$.

Preparation of Heavy and Light Polypeptide Chains. H and L chains were obtained after extensive reduction with 0.3 M β-mercaptoethanol in 6–7 M guanidine hydrochloride, alkylation with iodoacetamide, and filtration through Sephadex G-200 in 4 M guanidine hydrochloride (Small and Lamm, 1966).

Antisera. Rabbit anti-F(ab')$_2$ was prepared by

[1] Abbreviations used: IgG, γG-immunoglobin; IgM, γM-immunoglobulin; IgA, γA-immunoglobulin; DNP-BGG, dinitrophenylbovine γ-globulin; PBS, sodium phosphate buffered saline; TPCK, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone.

[2] These estimates of γ_1-globulin purity may well be on the low side since the solutions measured were made by dissolving lyophilized samples, and γ_1 is more difficult than γ_2 to solubilize under these conditions.

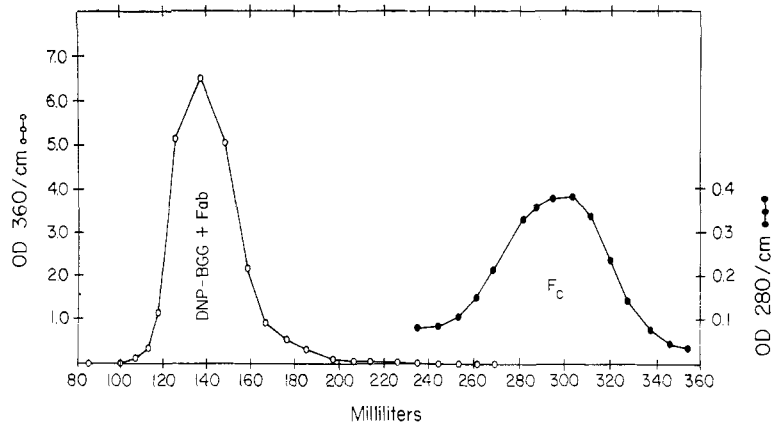

FIGURE 1: Separation of Fcγ_2 and unbound Fabγ_2 from DNP-BGG–Fabγ_2 complexes and free DNP-BGG by filtration through Sephadex G-200. The DNP-BGG is monitored by the absorption of the DNP groups at 360 mμ, and the Fc fragments are followed by their absorption at 280 mμ.

injecting F(ab')$_2$ fragments of guinea pig γ_2-globulin, in complete Freund's adjuvant, into the footpads (Nussenzweig and Benacerraf, 1966). Antisera directed specifically against the Fc fragment of γ_1- or γ_2-globulin were prepared as follows. (a) (anti-Fcγ_1) Rabbits were injected with the purified γ_1-globulin fraction obtained as described above from anti-DNP antibodies. γ_1-Globulin (2 mg) in complete adjuvant was injected into the footpads. Several bleedings were taken after 6 weeks, and the sera were absorbed with purified γ_2-globulin. (b) (anti Fcγ_2) These antisera were obtained by immunizing rabbits with purified normal γ_2-globulin. The animals were injected into the footpads with 1 mg of protein in complete adjuvant, and were bled 6 weeks later. The serum was absorbed with F(ab')$_2$ fragments of γ_2-globulin.

The rabbit antisera against Fcγ_1 and Fcγ_2 were tested for specificity by precipitation reactions with purified γ_1- and γ_2-globulins labeled with ^{131}I. The reactions were performed in the antibody excess zone, and the maximum amount of each labeled antigen which could be precipitated separately by both antisera was evaluated. The anti-Fcγ_1 antiserum precipitated approximately 85% of the purified γ_1-globulin preparation and 0–1% of the γ_2 preparation. The anti-Fcγ_2 antiserum precipitated approximately 95% of the γ_2-globulin preparation and 10% of the purified γ_1-globulin preparation. (This 10% precipitation is due to contaminating γ_2-globulins which are present to some degree in all our preparations of γ_1-globulin.)

Peptide mapping was performed according to the method of Katz *et al.* (1959). Approximately 3 mg of protein, after extensive reduction, alkylation, thorough dialysis against water, and lyophilization was digested with TPCK-treated trypsin (trypsin treated by the method of Kostka and Carpenter (1964) to inhibit chymotryptic activity) for 24 hr at room temperature (1% added initially and again at 8 hr) in 1% NH_4HCO_3. The digests, which usually varied from clear to opalescent but which sometimes contained suspended material, were applied to Whatman No. 3 paper and chromatographed for about 24 hr in 1-butanol–acetic acid–water. Electrophoresis was performed in pyridine–acetate buffer (pH 3.6) for 1 hr at 3300 v. Finally, the papers were stained with ninhydrin in ethanol–collidine–acetic acid.

Dialysis after reduction in guanidine could result in a loss of peptides if prior enzymatic digestion had cleaved peptide bonds between the half-cystines of an intrachain disulfide bond (Small *et al.*, 1966). To explore this possibility aliquots of pepsin and papain-treated protein were extensively reduced and then filtered through Sephadex G-200 in guanidine; significant amounts of tyrosine and tryptophan-containing peptides small enough to be lost during dialysis were not detected by absorption at 280 mμ.

Other Methods. Sedimentation coefficients were measured in the Spinco Model E ultracentrifuge at 59,780 rpm. Double diffusion in agar gels was performed according to Ouchterlony (1953) and immunoelectrophoresis by a modification of Scheidegger's (1955) technique (Benacerraf *et al.*, 1964). Disc electrophoresis was carried out in 4% polyacrylamide gels at alkaline pH in the presence of urea (Davis, 1964; Reisfeld and Small, 1966).

Results

Characterization of Products. When examined by sedimentation velocity in the analytical ultracentrifuge the fragments produced by pepsin and papain digestion exhibited single symmetrical peaks. The F(ab')$_2$ fragments from γ_1-globulin at 5.3 mg/ml and from γ_2-globulin at 6.6 mg/ml both had an $s_{20,w}$ = 5.1 S. The papain digest of γ_1-globulin at 5.6 mg/ml had an $s_{20,w}$ = 3.5 S, and the papain digest of γ_2-globulin at 6.5 mg/ml had an $s_{20,w}$ = 3.4 S. A $\bar{V}$ = 0.73 was assumed for the corrections.

When tested with appropriate antisera by the Ouchterlony (1953) technique the Fab and F(ab')$_2$ fragments

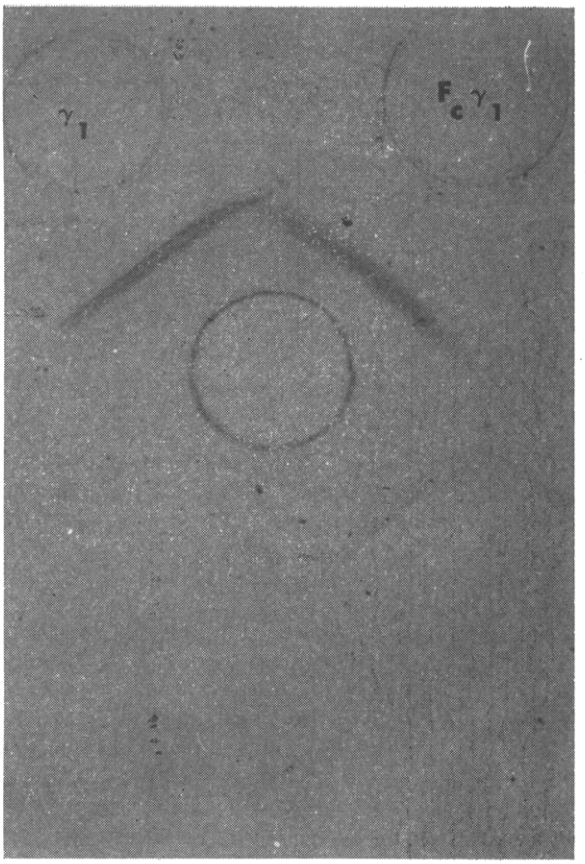

FIGURE 2: Fcγ_1 crystals, solubilized in sodium dodecyl sulfate, are antigenically deficient with respect to intact γ_1-globulin when tested with a rabbit antiserum against γ_1-globulin which had been absorbed with γ_2-globulin.

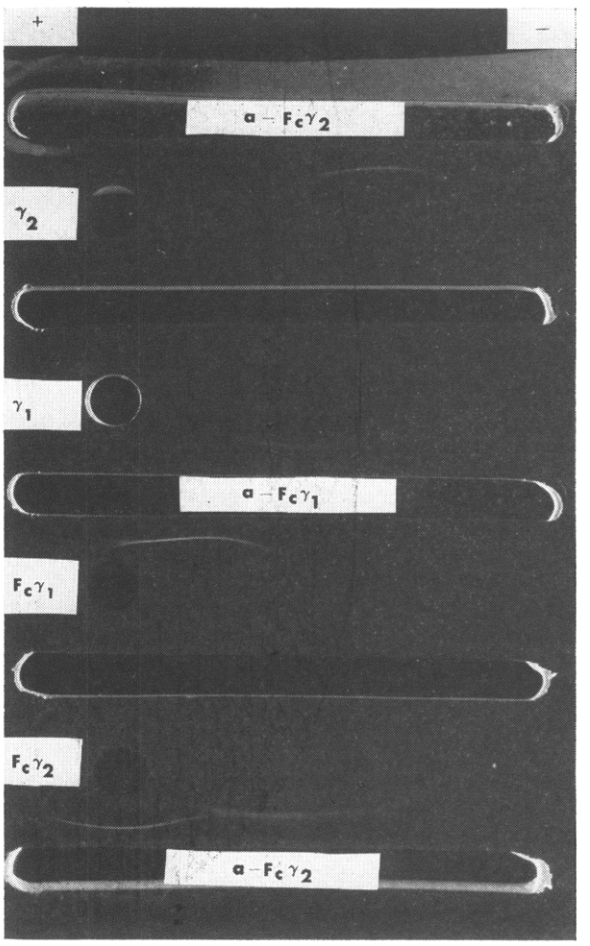

FIGURE 3: This immunoelectrophoresis experiment illustrates the differences in mobility between γ_1- and γ_2-globulins and their Fc fragments. The two upper wells contained intact molecules and the two lower wells papain digests thereof. The troughs contain rabbit antisera. In the bottom pattern the line closer to the anode is due to F′cγ_2, a further digestion product of the Fc fragment which is sometimes observed.

yielded reactions of partial identity with intact γ_1- and γ_2-globulins. The crystals derived from papain-treated γ_1-globulin are antigenically deficient with respect to the Fc portion of intact γ_1-globulin (Figure 2). The precipitation line of the latter spurs over the line due to the solubilized crystals when reacted with an anti-γ_1-globulin antiserum rendered specific for Fc_{γ_1} by absorption with γ_2-globulin. (The spur is not due to antigenic determinants in the $F(ab')_2$ fragment since these are identical in both classes.) Figure 3 illustrates the difference in electrophoretic mobility between γ_1- and γ_2-globulins and their Fc fragments.

An antiserum specific for the $F(ab')_2$ fragment of γ_2-globulin and known to detect determinants in the Fd fragment (Nussenzweig and Benacerraf, 1966) could not distinguish among Fab_{γ_1}, Fab_{γ_2}, $F(ab')_{2\gamma_1}$, and $F(ab')_{2\gamma_2}$ (Figure 4). This finding is in agreement with the earlier report that the Fd fragments of γ_1- and γ_2-globulins share antigenic determinants (Nussenzweig and Benacerraf, 1966).

The Fab and $F(ab')_2$ fragments obtained from γ_1- and γ_2-anti-DNP antibodies are not identical, however. Thus, the banding patterns of extensively reduced and alkylated $F(ab')_2$ fragments in polyacrylamide gels after disc electrophoresis in buffers containing urea are slightly different (Figure 5). Since the L chains of guinea pig γ_1- and γ_2-anti-DNP antibodies behave similarly during starch gel (Benacerraf *et al.*, 1964) and disc (Aron and Lamm, 1967) electrophoresis, it is probable that the slight differences observed in Figure 5 are due to the Fd fragments. Also, after immunoelectrophoresis the Fab and $F(ab')_2$ fragments of γ_1-globulin were closer to the anode than the respective fragments from γ_2-globulin.

Polypeptide Chain Structure of Guinea Pig γ_1- and γ_2-Globulins. From previous studies it is known that γ_1- and γ_2-globulins have sedimentation coefficients of about 7 S (Benacerraf *et al.*, 1963), that the H and L chains of γ_2-globulin are similar in size to the corresponding chains of rabbit IgG (Nussenzweig *et al.*, 1966) and that the percentage of L chains in guinea

pig γ_2-globulin and rabbit IgG is similar (Fleischman *et al.*, 1963; Small and Lamm, 1966; Lamm *et al.*, 1966). In the present study guinea pig γ_1-globulin was found to contain a similar percentage of L chains, based on the absorption at 280 mμ of the H and L chain peaks in the gel filtration patterns after extensive reduction. In order to determine the approximate molecular weight of H_{γ_1}, equal amounts of H_{γ_1} and H_{γ_2} were mixed and filtered together through Sephadex G-200 in guanidine. The resulting elution pattern contained a single symmetrical peak with a maximum at the same elution volume as H_{γ_1} or H_{γ_2} separately; this indicates that H_{γ_1} and H_{γ_2} are approximately the same size. L_{γ_1} and L_{γ_2} are also similar in size by the same criterion. It therefore follows that both these classes of guinea pig immunoglobulins conform to the model of Fleischman *et al.* (1963) for IgG.

Because γ_1-globulin is low in hexose, it is thought not to be analogous to the IgA immunoglobulins of other species (Oettgen *et al.*, 1965). Another physicochemical difference between IgG and IgA lies in the size of their H chains, the α chain being larger than the γ chain (Cebra and Small, 1967). This difference can be detected by different elution positions after gel filtration. Therefore, in order to confirm that γ_1-globulin is not the counterpart in guinea pigs to IgA, extensively reduced and alkylated bovine serum albumin, which is similar in size to an α chain, was also passed through the Sephadex column in guanidine. It eluted 20 ml sooner than did guinea pig H chains.

Peptide Mapping Studies. Comparisons between γ_1- and γ_2-globulins were facilitated by making composite maps along with maps of individual components. Thus, it was usually possible to decide whether two similar peptides in different maps were identical or not according to whether they appeared singly or doubly in the map of the mixture.

Peptide maps of Fab and Fc fragments and of L and H chains from γ_1- and γ_2-globulins are shown in Figures 6 and 7. All comparisons were repeated between one and three times, and the over-all results are summarized in Table I, which indicates the number of spots per map and how many were observed in one class of immunoglobulin and not the other. Fab and $F(ab')_2$ fragments (latter maps not shown) are very similar, L chains are identical, H chains have about half their spots in common, and Fc fragments are quite different.

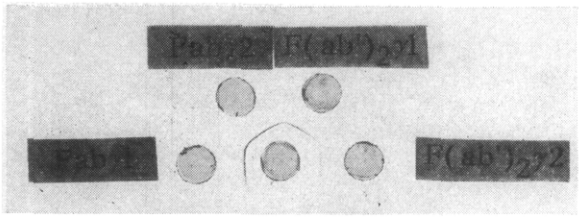

FIGURE 4: The Fab and $F(ab')_2$ fragments of γ_1- and γ_2-globulins give reactions of identity when tested with a rabbit antiserum prepared against the $F(ab')_2$ fragment of γ_2-globulin, and which contains antibodies directed against the Fd fragment.

The data in Table I should be interpreted in the knowledge that not all the spots in the H chain maps could be found in the respective Fc and Fab maps and *vice versa*. The possibility of being misled by technical artifacts related to variable proteolysis by papain and pepsin can be reduced by using for comparison only those H chain spots which are also found in the respective Fc or non-L parts of the Fab maps (Table II). This procedure was able to account for about 80% of the H chain spots. Approximately 18 and 26 peptides could be assigned to Fc_{γ_1} and Fc_{γ_2}, respectively. Of these, about ten were common to both. Similarly eight and nine peptides could be assigned to Fd_{γ_2} and Fd_{γ_1}, respectively. All eight of the Fd_{γ_2} peptides were present in Fd_{γ_1}, and eight of the nine Fd_{γ_1} peptides were found in Fd_{γ_2}.

Table I lists about 15 distinctive spots in H_{γ_1}, only 9 of which could be found in Fc_{γ_1} and Fd_{γ_1} (Table II). The most probable explanation is that part of the Fc_{γ_1} fragment is hydrolyzed by papain into small peptides

TABLE I: Summary of Peptide Mapping Experiments.

Component	Total No. of Spots	Distinctive Spots for γ_1	Distinctive Spots for γ_2
Fab	30–35[a]	1	2–3
$F(ab')_2$	35–40[a]	2–4	4–6
Fc	~30[a]	~20	~20
H	35–40	~15	15–20
L	20–30[b]	0	0

[a] These fragments were made by digestion with papain or pepsin. Some of the resultant peptides are probably technical artifacts (see text). [b] The number of L spots detected depends on the amount of hydrolysate applied to the paper. About 30 spots were obtained from 3 mg, and about 20 spots were obtained from 1.5 mg, which is approximately the amount of L chain peptides in the Fab and $F(ab')_2$ maps.

TABLE II: Comparison of H Chain Peptides of γ_1- and γ_2-Globulins Which Can Be Assigned to the Fc and Fd Portions.[a]

Polypeptide Chains	H Chain Spots Found in Fc		H Chain Spots Found in Fd	
	Total	Specific	Total	Specific
$H\gamma_1$	18	8	9	1
$H\gamma_2$	26	16	8	0

[a] Data from one set of maps.

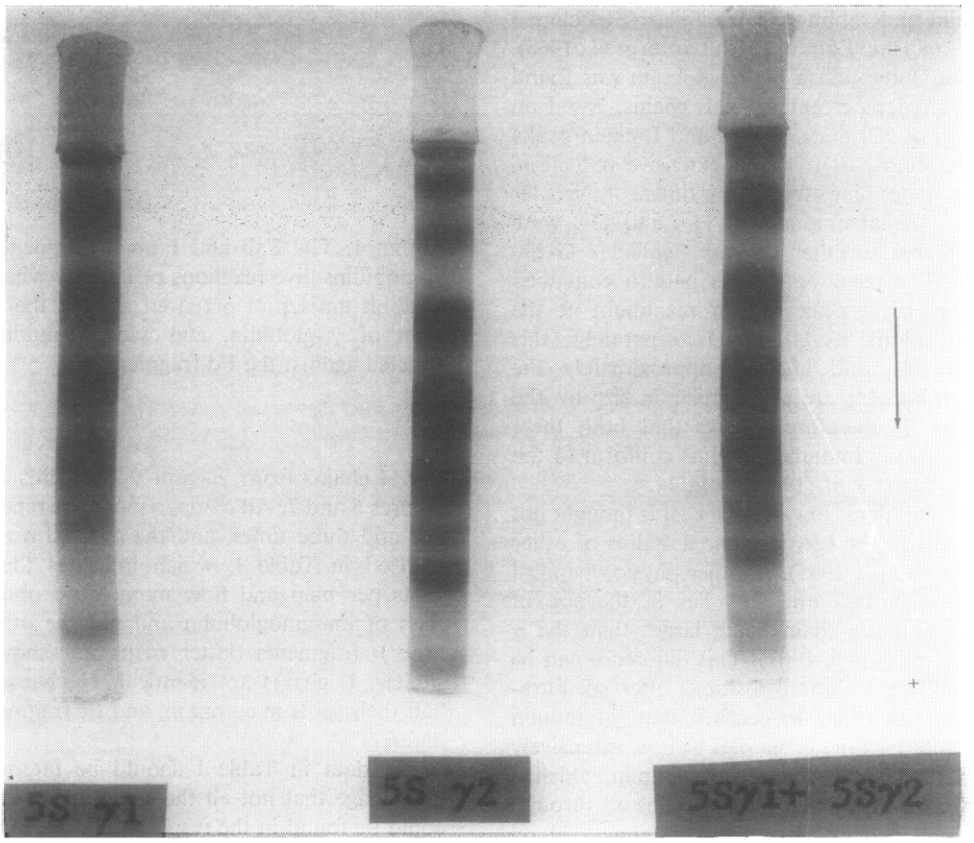

FIGURE 5: Banding patterns of extensively reduced and alkylated F(ab′)$_2$ fragments after disc electrophoresis in polyacrylamide gels with urea-containing buffers.

which are lost during subsequent dialysis. This is consistent with the finding that the Fc$_{\gamma_1}$ crystals contain fewer antigenic determinants than expected. In contrast, the distinctive H$_{\gamma_2}$ spots listed in Table I can be accounted for in the Fc$_{\gamma_2}$ maps (Table II).

In order to rule out the possibility that the marked similarities between Fab and F(ab′)$_2$ fragments of γ_1- and γ_2-globulins were due to a directing influence of the DNP-BGG antigen used for immunization, maps of F(ab′)$_2$ fragments and H chains from γ_1- and γ_2-anti-DNP antibodies were compared with the same components obtained from nonspecific, normal γ_2-globulin with results similar to those described above.

Discussion

In these experiments tryptic digests of the fragments and polypeptide chains of guinea pig γ_1- and γ_2-globulins have been compared by means of peptide mapping. The Fab and F(ab′)$_2$ fragments of these two classes of immunoglobulins are very similar, their L chains are identical, the H chains share about half their peptides in common, and the Fc fragments are quite different, although they do share about ten peptides. Apparently the Fd fragments of γ_1- and γ_2-globulins resemble each other closely, and the major differences between the two classes are confined to the Fc fragments.

Frangione and Franklin (1965a) studied the Fd fragments of human normal and myeloma IgG proteins by comparing H chain and Fc fragment peptide maps. Their results indicated the presence of constant and variable regions in the Fd fragment. This concept is supported by other studies of H chains (Frangione and Franklin, 1965b; Bernier *et al.*, 1965). In the present work heterogeneous antibodies were investigated, and therefore only constant portions of the Fd fragments would be expected to provide detectable amounts of peptides. How much of the Fd fragment is thus represented is not known.

The extent of class differences among Fc fragments in a given animal species as revealed by peptide mapping can vary widely, *e.g.*, the findings of Potter *et al.* (1966) and Lieberman and Potter (1966) in the mouse. For both humans and rabbits peptide maps of α, γ, and μ chains are quite different (Bernier *et al.*, 1965; Frangione and Franklin, 1965b; Lamm and Small, 1966; Cebra and Small, 1967), and the same therefore probably holds for the corresponding Fc fragments. The four subclasses of human IgG contain similar Fc fragments (Frangione *et al.*, 1966).

Classes of immunoglobulins have been traditionally distinguished from subclasses by having H chains

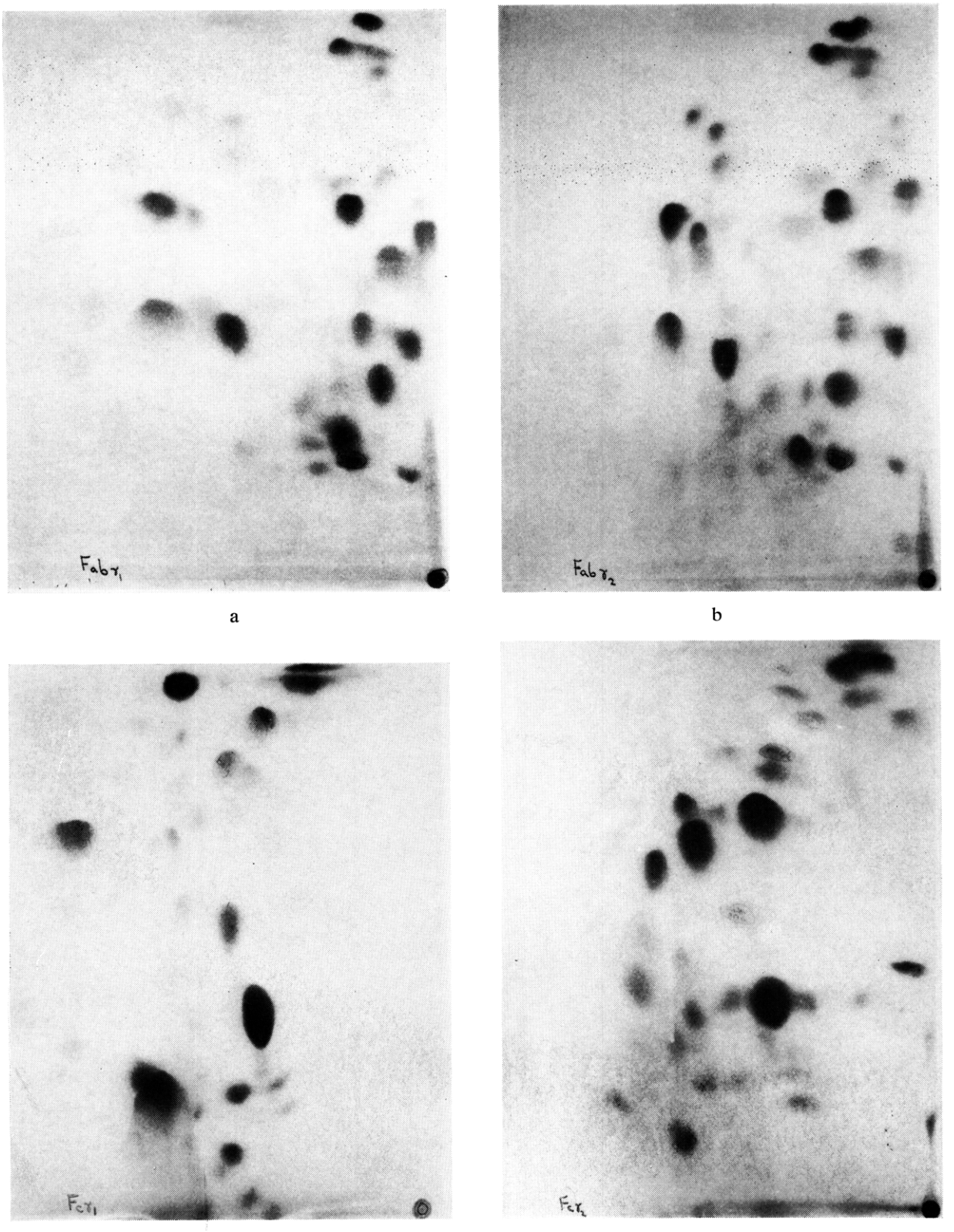

FIGURE 6: Peptide maps of Fab and Fc fragments. In these maps and in those of Figure 7 the sample was applied at the lower right corner, chromatography was from right to left, and electrophoresis was from bottom to top.

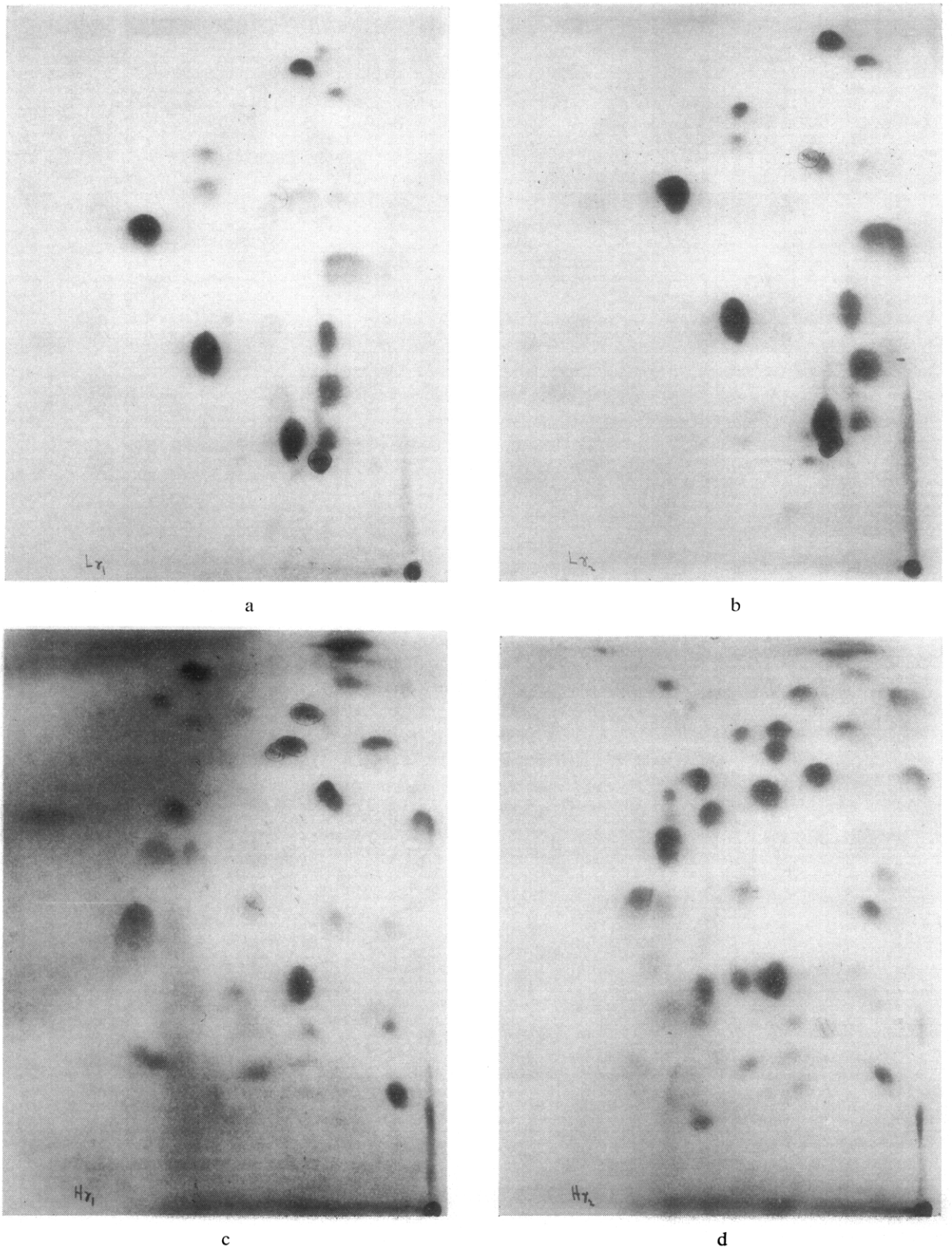

FIGURE 7: Peptide maps of L and H chains.

which do not cross react antigenically and which mediate different biological phenomena. The first criterion no longer holds for the Fd part of the H chain. Therefore, the distinction must be based solely on Fc differences. Guinea pig γ_1- and γ_2-globulins are considered to be distinct classes because their Fc-fragments cross react minimally, if at all, with rabbit antisera (V. Nussenzweig, unpublished data), yield different peptide maps, and mediate different biological phenomena (Ovary *et al.*, 1963; Bloch *et al.*, 1963; Berken and Benacerraf, 1966). The H chains of γ_1- and γ_2-globulins appear, however, to be more closely related than γ, α, and μ chains because their size and carbohydrate content are similar and they share about half their tryptic peptides in common.

Since the H chains of guinea pig γ_1- and γ_2-globulins have so many common peptides, it seems reasonable to postulate that they arose *via* gene duplication. With the passage of time major differences could have developed in the Fc portions as the result of mutations, with only relatively minor changes in the Fd portions. However, it is also possible that the differences observed in the peptide maps and in the electrophoretic mobilities of Fab and $F(ab')_2$ fragments of γ_1- and γ_2-globulins are technical artifacts and that the Fd fragments are truly identical. Such a situation could arise for a number of reasons. For example, the points of cleavage by papain and pepsin could differ even in a region which had the same amino acid sequence in both γ_1- and γ_2-globulins if steric factors at the site of enzyme action were dissimilar as a result of the different Fc fragments in the two classes. In the case of pepsin, another factor might be the different pH values used to digest γ_1- and γ_2-globulins. Also, because pepsin probably splits closer to the C-terminal end of the H chain than papain, it is likely that the $F(ab')_2$ fragment contains a small portion of the Fc fragment. Thus, even if the Fd fragments were identical, the $F(ab')_2$ fragments could differ by a few peptides. This conjecture is consistent with the Fab maps being more similar than the $F(ab')_2$ maps. Finally, artifacts could occur if papain and pepsin act at a variety of sites within the same general region of the H chain rather than being confined to a specific peptide bond. Evidence for this phenomenon has been obtained with rabbit Fc fragments (Hill *et al.*, 1966). By this means a number of different tryptic peptides could result from the same primary amino acid sequence. This supposition is consistent with there being a number of spots in the Fc and Fab maps that could not be found in the H chain maps and with the observed antigenic deficiency of Fc_{γ_1} crystals as compared to the Fc region in intact γ_1-globulin when tested with an anti-γ_1-globulin antiserum previously absorbed with γ_2-globulin. Since pepsin probably splits the H chain closer to the C-terminal end than papain and since the $F(ab')_2$ portion of the γ_2-globulin used to absorb the anti-γ_1-antiserum is indistinguishable antigenically from $F(ab')_{2\gamma_1}$, this absorbed antiserum might be expected to detect determinants in only a portion of the Fc fragment of intact γ_1-globulin. It was therefore surprising that the $Fc_{\gamma 1}$ crystals were deficient. The Fc_{γ_1} crystals also lack the ability of intact γ_1-globulin to fix to receptor sites in guinea pig skin (Nussenzweig and Benacerraf, 1964). In summary, the crystals may therefore represent only a portion of the total Fc fragment. Of course, it is entirely possible that the Fc_{γ_1} crystals are deficient because they have a different conformation from the Fc portion of intact γ_1-globulin.

If the Fd fragments are identical in γ_1- and γ_2-globulins, the possibility should be entertained that the Fc spots common to both classes reflect a sequence located in the part of the Fc fragment adjacent to the Fd fragment. According to this idea there would be a region with a common primary structure in both γ_1- and γ_2-globulins extending from the amino-terminal end of the H chain (or from the beginning of a postulated constant portion of the Fd fragment) part way into the Fc portion. On the other hand, the fact that the Fab fragments appear to be more similar than the $F(ab')_2$ fragments is more consistent with there being differences in the portion of the Fc fragment adjacent to Fd.

From the data obtained by peptide mapping and by antigenic analysis with appropriate antisera it appears likely that the portion of the molecule concerned with the recognition of and combination with antigen is basically similar in guinea pig γ_1- and γ_2-globulins and that the opposite situation applies to the portion of the molecule concerned with biological activity. These findings are consistent with there being a selective evolutionary pressure tending to conserve the structure of the Fab fragment, whose function is the same in different immunoglobulin classes. Such a selective pressure would not necessarily be expected to operate on the portion of the molecule concerned with biological activity, and, indeed, it seems not unreasonable to suspect that selective pressures on the Fc portion might be in the opposite direction, *i.e.*, there might be survival value in antibodies being able to mediate a number of biological functions. Evolutionary diversion from the original Fc precursor could well occur to different extents in different animal species. This would be consistent with the observed variation throughout the animal kingdom in the number of classes and subclasses of antibodies and their biological activities.

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Added in Proof

van Dalen *et al.* (1967) have reported that rabbit γ and μ chains share about half their tryptic peptides in common.

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