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# Heterogeneity of the Peptide Chains of γ-Globulin

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When γ-globulin is reduced in neutral aqueous solution and dialysed against acetic acid or propionic acid, the molecule dissociates into two types of peptide chain (A and B); these can be separated by gel-filtration (Fleischman, Pain & Porter, 1962) and retain several biological properties of the original molecule. The A chains comprise about three-quarters of the molecule, have a molecular weight of about 50000 (Pain, 1963), are associated with the antibody-combining sites of horse antibodies (Fleischman, Porter & Press, 1963), and carry the antigenic (Cohen, 1963*a*, *c*) and allotypic (S. Lawler, personal communication; see Cohen, 1963*a*) determinants specific for each type of γ-globulin. The B chains have a molecular weight of 20000 (Pain, 1963) and carry antigenic and allotypic determinants common to all types of γ-globulin (Cohen, 1963*a*).

The heterogeneity of both A and B chains is apparent from the antigenic determinants and antibody-combining sites that they carry. Similarly, the *N*-terminal amino acids of the chains are complex in all species that have been examined (Fleischman *et al.* 1962; Cohen, 1963*c*). Further evidence of the complexity of the A chains is described in the present paper and, in particular, the B chains from several species are shown to exist in some eight to ten electrophoretically distinct forms. A brief account of this work has been given by Cohen (1963*b*).

## MATERIALS

*γ-Globulin preparations.* Normal 7s and $\gamma_1$M-globulins and pathological 7s, $\gamma_1$A- and $\gamma_1$M-globulins were prepared as described by Cohen (1963*c*). Subfractions of normal 7s γ-globulin were prepared by chromatography on carboxymethylcellulose according to the method described by Sober & Peterson (1958). Serum taken from a colostrum-deprived calf 19 days after birth was fractionated by precipitating twice with $Na_2SO_4$ (final concn. 18%, w/v). This precipitate, which contained an $\alpha_2$-globulin (fetuin) and γ-globulin, was dialysed against water at 2°. The euglobulin precipitate was washed in water and dissolved in 0·2M-boric acid buffer, pH 8·2; this fraction had the mobility of γ-globulin on paper electrophoresis, and in the ultracentrifuge contained a main peak (having an $S_{20}$ value of 6·8s) comprising about 85% of the total and three minor components having $S_{20}$ values of 3·3s, 14s and 21s. The yield of γ-globulin was approx. 30 mg./100 ml. of serum. Samples of γ-globulin from the same colostrum-deprived calf at 40 and 67 days were prepared by chromatography on diethylaminoethylcellulose (Sober & Peterson, 1958); the yields of γ-globulin were 130 and 200 mg./100 ml. respectively. A euglobulin fraction of adult bovine γ-globulin was prepared from serum dialysed against 0·0175M-sodium phosphate buffer, pH 6·4. The euglobulin precipitate was washed in water, dissolved in 0·9% NaCl and precipitated three times with $Na_2SO_4$ (final concn. 18%, w/v).

*Preparation of antisera.* Antisera to human γ-globulin and Bence-Jones proteins were obtained by immunizing rabbits with subcutaneous injections of the antigens (1–3 mg. of protein/week for 3–6 weeks) in Freund's complete adjuvant. Antisera to ovalbumin and bovine

serum albumin were raised in guinea pigs; the proteins in complete adjuvant were injected into the four foot-pads (total 5 mg. of protein in 0·25 ml.) and the animals bled after 4–6 weeks.

*Isolation of antibody precipitates.* Guinea-pig sera were decomplemented by heating at 60° for 30 min. Anti-ovalbumin and anti-(bovine serum albumin) were precipitated at equivalence and washed three times in cold 0·9% NaCl.

## METHODS

*Reduction and isolation of A and B chains.* Normal and pathological γ-globulins were reduced and fractionated on Sephadex G-75 by methods described by Fleischman *et al.* (1962). Guinea-pig antibody precipitates were suspended in 1M-tris buffer, pH 8·2, and made 0·95M with respect to β-mercaptoethanol. The suspensions were shaken for 2 hr. at room temperature, then cooled in ice, and an equal volume of 0·95M-iodoacetamide (recrystallized) was added and the pH kept at about 8 by addition of trimethylamine. After about 1 hr. the suspensions were dialysed against 100 vol. of cold 0·9% NaCl and then overnight against N-acetic acid. The reduced precipitates were now in clear solution and were fractionated on Sephadex G-75 columns in N-acetic acid. The antigen remained with the A chain under these conditions.

*Electrophoresis.* Starch-gel electrophoresis was carried out in vertical trays by using: (i) 8M-urea and 0·05M-formic acid as described by Edelman & Poulik (1961); (ii) 8M-urea and 0·035M-glycine buffer, pH 8·8; the final pH of the gels was 7–8. Electrode vessels contained 0·3M-boric acid–0·06N-NaOH, pH 8·2. Electrophoresis was continued for 15–24 hr. at 5v/cm. Samples for gel electrophoresis were run at concentrations of 3–12 mg./ml. Electrophoresis in acrylamide gels prepared according to the method of Ornstein (1962) was carried out in vertical trays by using 6·5% (w/v) acrylamide in 8M-urea and 0·05M-tris buffer, pH 8·8.

*Gel diffusion.* Double-diffusion in agar was carried out as described by Ouchterlony (1953). Myeloma proteins were classified as 7S or $\gamma_1$A-globulins by using antisera absorbed so that they reacted specifically with each kind of protein. The isolated B chains of myeloma proteins were classified into antigenic types I and II on the basis of cross-reactions with Bence-Jones proteins of known type by using rabbit antisera to human 7S γ-globulin or antisera to Bence-Jones proteins. The classification into antigenic types I and II corresponds to the grouping described by Korngold & Lipari (1956).

*N-Terminal analysis.* *N*-Terminal amino acids were determined by the fluorodinitrobenzene method of Sanger (Porter, 1957).

*Amino acid analysis.* This was carried out in a Spinco Amino Acid Analyzer on single samples hydrolysed for 24 hr. under the conditions described by Crumpton & Wilkinson (1963).

## RESULTS

*Electrophoresis of A chains.* In urea–glycine starch gels, pH 7–8, the A chain of human γ-globulin migrates as a diffuse band showing approximately the same spread as the original γ-globulin. The A chains from $\gamma_1$ and $\gamma_2$ subfractions of 7S γ-globulin are somewhat less heterogeneous and have the same relative mobilities as the proteins from which they are prepared (Fig. 1). The A chains isolated from myeloma γ-globulins also show the same relative heterogeneity and mobility as their parent proteins when analysed on acrylamide gels in urea–tris buffer, pH 8·8 (Fig. 2).

*Electrophoresis of B chains.* The B chain of human γ-globulin appears as a diffuse band on electrophoresis in urea–formic acid starch gels (Edelman & Poulik, 1961). However, electrophoresis in urea–glycine starch gels, pH 7–8, resolved human B chains into ten distinct components, three of which move towards the cathode and seven towards the anode. These subfractions have been numbered $B_1$ to $B_{10}$ from the cathodal end of the gel (Fig. 3). The B chains isolated from normal 7S and $\gamma_1$M-globulins appear to be identical (Fig. 3). There was no correlation between the mobility of the whole γ-globulin and that of the B chains derived from it, as B chains from fast-moving and slow-moving γ-globulins were indistinguishable (Fig. 3). Similarly, the B chains from euglobulin and pseudoglobulin subfractions of γ-globulin were identical. Individual bands could be eluted from a starch gel, and when re-run under the same conditions they behaved as single components with the same mobilities as shown originally (Fig. 4). When subjected to electrophoresis in urea–formic acid gels they remained homogeneous with mobilities within the range of the whole B chains.

It seemed, therefore, that the multiplicity of components was not an artifact of the electrophoresis, but it could have been caused by the methods used to isolate the B chains. There were several possibilities, e.g.: (i) unequal reduction of B chains; (ii) variable hydrolysis of amide groups during fractionation in N-acetic acid; (iii) reaction of amino groups with cyanate which is in equilibrium with urea at neutral and alkaline pH (Stark, Stein & Moore, 1960). However, the electrophoretic behaviour of B chains was not altered by more complete reduction with mercaptoethanol in the presence of 6M-urea, or by exposure to N-acetic acid for 4 weeks at 2°; the B chains were also unaltered after being left for 5 days in 8M-urea previously heated to 70° for 10 min. in a glycine buffer, pH 9.

The samples of human B chain shown in Fig. 3 were isolated from pooled γ-globulin; the observed heterogeneity could therefore have resulted from differences in the patterns of B chains among individuals. However, B chains isolated from 7S γ-globulins of three Caucasian, two Chinese and three African adults were all identical on electrophoresis in urea–glycine starch gels. In addition, the B chains obtained from three sets of parents of

Table 1. *Some properties of pathological serum proteins and their B chains*

The clinical diagnoses were: M, myelomatosis; C, cryoglobulinaemia; I, idiopathic hyper-$\gamma$-globulinaemia; Mac, macroglobulinaemia. Bence-Jones proteinuria was detected by the heat test on concentrates of urine at pH 5. The $\gamma$-globulin grouping was based on reaction with specific antisera (see the Methods section) and ultracentrifuging. The antigenic type was determined by comparison with types I and II Bence-Jones proteins (see the Methods section). The electrophoretic mobilities of the B chains in urea–glycine starch gel are given as numbers indicating the mobilities of corresponding normal B fractions (see Figs. 3 and 7).

| | | | $\gamma$-Globulin | | | B chain | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Electrophoretic mobility | | *N*-Terminal amino acid (moles/20000 g.) | |
| Case | Clinical diagnosis | Bence-Jones proteinuria | Group | Antigenic type | Electrophoretic mobility | Antigenic type | Main fraction | Minor fraction | Asp | Glu |
| Kou/4 | M | + | 7s$\gamma$ | I | $\gamma_2$ | I | $B_5$ | $B_6$ | 0 | 0·7 |
| Ink/5 | M | − | 7s$\gamma$ | II | $\gamma_2$ | II | $B_3$ and $B_4$ | $B_1$ | 0 | Trace |
| Ste/6 | M | + | 7s$\gamma$ | II | $\gamma_1$ | II | $B_3$ | $B_1$ | 0 | 0 |
| Bro/38 | M | + | 7s$\gamma$ | I | $\gamma_1$ | I | $B_6$ | $B_7$ | 0 | 0·3 |
| Low/44 | M | − | 7s$\gamma$ | I | $\gamma_2$ | I | $B_4$ | $B_5$ | 0 | 0·2 |
| She/52 | M | + | 7s$\gamma$ | I | $\gamma_2$ | I | $B_4$ | $B_5$ | 0 | 0·4 |
| Mit/29 | M | − | $\gamma_1$A | II | $\beta$ | II | $B_3$ | $B_4$ | 0 | 0 |
| Fie/9 | C | − | 7s$\gamma$ | I | $\gamma_2$ | I | $B_5$ | $B_6$ | 0·3 | 0 |
| Dra/8 | I | − | 7s$\gamma$ | I and II | $\gamma_2$ | I and II | $B_4$ | $B_5$ | 0 | 0 |
| Daw/28 | I | − | 7s$\gamma$ | II | $\gamma_1$ | II | $B_4$ | $B_5$ | 0 | 0 |
| Wat/88 | Mac | − | $\gamma_1$M | I | $\gamma_1$ | I | $B_1$ | $B_1$ | 0 | 0·3 |
| Gre/83 | Mac, C | − | $\gamma_1$M | II | $\gamma_1$ | II | $B_5$ | $B_6$ | 0 | 0·1 |

a-$\gamma$-globulinaemic children were also indistinguishable from normal B chains.

A similar degree of heterogeneity was observed when B chains from other species were examined; thus B chains isolated from rabbit, guinea-pig, bovine, horse and baboon $\gamma$-globulins all showed multiple components, although their number and mobility varied in different species (Fig. 5). Rabbit $\gamma$-globulin B chain was more acidic than B chains of other species and the banding was sharper when electrophoresis was carried out at pH 5–6.

*B chains of specific antibodies.* The B chains separated from guinea-pig antibody–antigen precipitates contained all the components of normal B chains when analysed in a urea–glycine starch gel, pH 8 (Fig. 6), although there appeared to be differences in the relative intensities of B-chain bands in different antibodies. Thus in two preparations of anti-ovalbumin, from different sera, subfractions $B_2$, $B_3$, $B_4$ and $B_7$ were relatively prominent, whereas in anti-(bovine serum albumin) subfractions $B_3$, $B_4$ and $B_5$ stained with greatest intensity (Fig. 6).

*B chains of myeloma proteins.* Poulik & Edelman (1961) have shown that the B chains of myeloma $\gamma$-globulins are homogeneous as compared with normal B chains on electrophoresis in urea–formic acid gels. The relative homogeneity of myeloma B chains is equally striking when electrophoresis is carried out at alkaline pH. In acrylamide gels, pH 8·8, normal B chains show a broad electrophoretic spread whereas myeloma B chains appear as sharp bands; the relative mobility of patho-

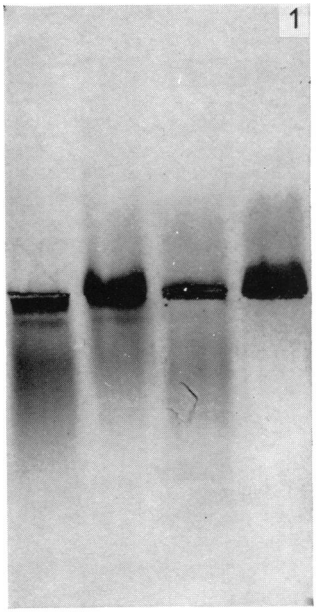
## EXPLANATION OF PLATE 1

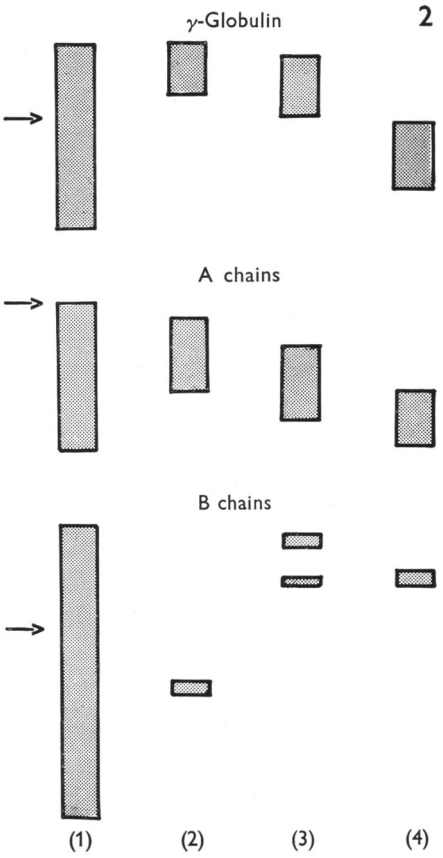
Fig. 1. Electrophoresis in 8M-urea–glycine starch gel of $\gamma_1$ (1) and $\gamma_2$ (2) subfractions of normal human 7s $\gamma$-globulin and the A chains of these $\gamma_1$ (3) and $\gamma_2$ (4) fractions.

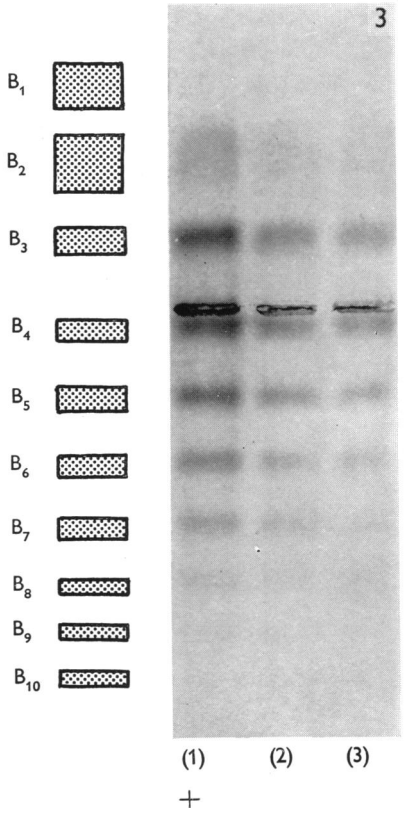
Fig. 2. Electrophoresis of whole $\gamma$-globulin and of the A and B chains of human 7s $\gamma$-globulin (1) and of three myeloma $\gamma$-globulins: Kou/4 (2), Ink/5 (3) and Ste/6 (4) (see Table 1). Arrows indicate the points of insertion of the samples; the anode is at the bottom in each case. Whole $\gamma$-globulin and A chains were analysed in 8M-urea–tris acrylamide gels, pH 8·8; B chains were analysed in acrylamide gels containing tris buffer, pH 8·8.

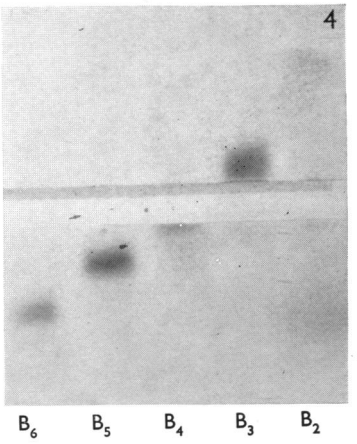
Fig. 3. Electrophoresis in 8M-urea–glycine starch gel of the B chains from $\gamma_1$'(1) and $\gamma_2$ (2) subfractions of normal human 7s $\gamma$-globulin (see the Methods section) and from normal human $\gamma_1$M-globulin (3). A diagrammatic representation of the ten components of the human B chain is shown on the left; these subfractions have been designated from the cathodal end as $B_1$ to $B_{10}$.

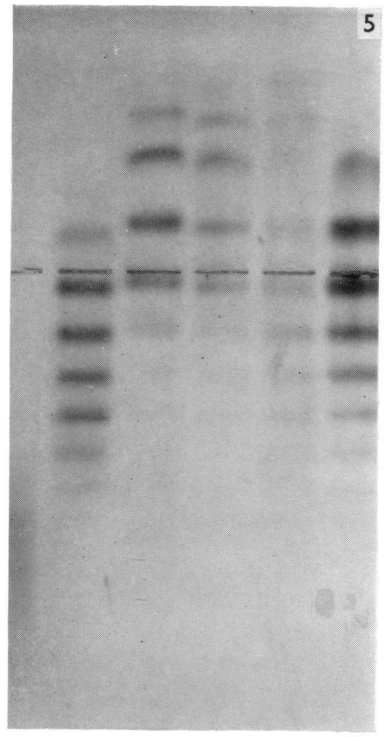
Fig. 4. Electrophoresis in 8M-urea–glycine starch gels of subfractions ($B_2$–$B_6$) of normal human B chains. These components were separated by electrophoresis in a urea–glycine starch gel, cut out and inserted into the 1 cm. slot shown and again subjected to electrophoresis.

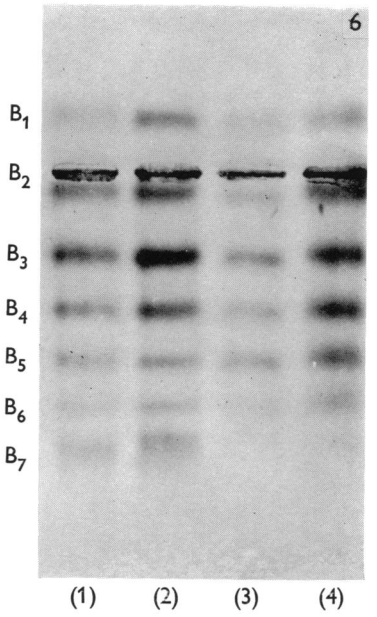

1
(1)
(2)
(3)
(4)

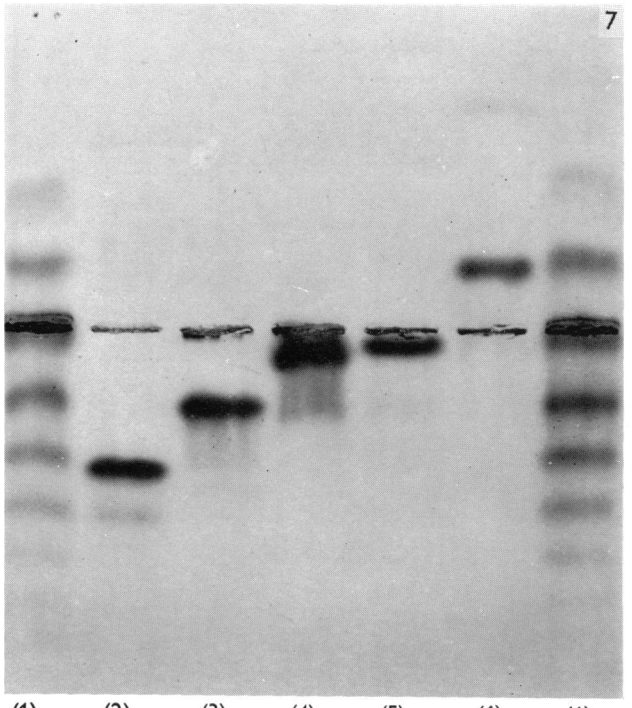

γ-Globulin
2
A chains
B chains
(1)
(2)
(3)
(4)


−
3
B1
B2
B3
B4
B5
B6
B7
B8
B9
B10
(1)
(2)
(3)
+


4
B6
B5
B4
B3
B2



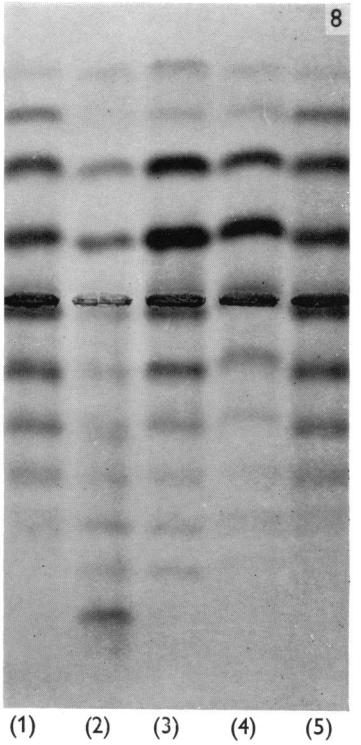




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Table 2. *Amino acid analysis of human normal B chains and of pathological B chains of different antigenic types and different electrophoretic mobilities*

The samples were taken from the appropriate cases given in Table 1. The antigenic type is that corresponding to Bence-Jones proteins types I and II (see the text). The electrophoretic components (in urea–glycine starch gel, pH 8) are as shown in Fig. 3. The amino acid contents are given on the basis of a mol.wt. of 20000 for B chain.

| | Amino acid content (moles/mole) | | | | |
|---|---|---|---|---|---|
| Sample ... ... ... ... | Normal | Bro/38 | She/52 | Daw/28 | Gre/83 |
| Antigenic type ... ... ... | I+II | I | I | II | II |
| Electrophoretic components ... | $B_1$–$B_{10}$ | $B_6$ ($B_7$) | $B_4$ ($B_5$) | $B_4$ ($B_5$) | $B_5$ ($B_6$) |
| Lys | 10 | 11 | 11 | 11 | 9·8 |
| His* | 2·9 | 2·1 | 2·0 | 2·2 | 2·1 |
| Arg | 6·3 | 5·3 | 5·1 | 3·9 | 5·4 |
| Asp | 14 | 17 | 14 | 13 | 15 |
| Thr* | 16 | 16 | 16 | 19 | 18 |
| Ser* | 26 | 22 | 28 | 28 | 24 |
| Glu | 22 | 23 | 23 | 17 | 17 |
| Pro | 11 | 11 | 10 | 16 | 16 |
| Gly | 13 | 11 | 10 | 18 | 14 |
| Ala | 13 | 11 | 11 | 15 | 14 |
| Val* | 14 | 12 | 11 | 15 | 15 |
| Met | 0·7 | 0·9 | 0·9 | 0 | 1·1 |
| Ileu | 5·2 | 5·5 | 6·3 | 5·4 | 5·5 |
| Leu | 14 | 14 | 14 | 14 | 13 |
| Tyr* | 8·2 | 9·7 | 8·8 | 8·6 | 7·7 |
| Phe | 6·5 | 5·9 | 7·7 | 3·8 | 4·5 |
| CyS* | 3·1 | 3·9 | 4·0 | 3·0 | 3·3 |
| $CyS{\cdot}CH_2{\cdot}CO_2H$ | 0·9 | 1·5 | 0·9 | 0·8 | 2·0 |
| Try | 2·3 | 1·9 | 1·4 | 3·6 | 4·3 |

* Based on approximate corrections for destruction or incomplete hydrolysis (Crumpton & Wilkinson, 1963).

logical B chains is unrelated to that of the original γ-globulin (Fig. 2). When examined in urea–glycine gels, pH 7–8, B chains isolated from 12 pathological γ-globulins (Table 1) all consisted of a single major component, usually with a second minor component (Fig. 7). In 11 cases the pathological B chains corresponded in mobility to one or other of the normal components of B (Table 1); the pathological B chains examined had components with mobilities corresponding to six out of the ten subfractions of normal B chains. In one case (Table 1; Wat/88) the main B-chain component from a pathological $\gamma_1$M-globulin had a mobility slower than that of the normal $B_1$ fraction.

The antigenic determinants that correspond to Bence-Jones proteins of types I and II are common to all γ-globulins (Mannik & Kunkel, 1963) and are present on the B chain (Cohen, 1963*c*). Pathological B chains, in the majority of instances, are either type I or type II, and this antigenic specificity is the same as that of the original γ-globulin. There is no consistent correlation between antigenic type and electrophoretic mobility of pathological B chains; for example, of the three pathological B chains that had the mobility of fraction $B_5$, two were antigenic type I and one was type II (Table 1 and Fig. 7: cf. samples 4 and 5).

Amino acid analyses on single samples hydrolysed for 24 hr. show differences between myeloma B chains of different mobilities and more especially between B chains of different antigenic type (Table 2). *N*-Terminal-amino acid analysis has given variable results with different pathological B chains; in some no *N*-terminal amino acid was detectable, whereas either aspartic acid or glutamic acid was present in others (Cohen, 1963*c*). It is apparent from the results given in Table 1 that the results of *N*-terminal-amino acid analysis cannot be consistently correlated with the electrophoretic mobility of pathological B chains.

## EXPLANATION OF PLATE 2

Fig. 5. Electrophoresis in 8M-urea–glycine starch gel of 7S γ-globulin B chains of rabbit (1), guinea pig (2), bovine (3), horse (4), baboon (5) and human (6).

Fig. 6. Electrophoresis in 8M-urea–glycine starch gel of the B chains of two different preparations of guinea-pig anti-ovalbumin (1 and 2), guinea-pig anti-(bovine serum albumin) (3) and guinea-pig γ-globulin (4).

Fig. 7. Electrophoresis in 8M-urea–glycine starch gel of the B chains of normal human 7S γ-globulin (1) and of five pathological γ-globulins: Bro/38 (2), Kou/4 (3), Low/44 (4), Daw/28 (5) and Ste/6 (6) (see Table 1).

Fig. 8. Electrophoresis in 8M-urea–glycine starch gel of the B chains of γ-globulin from a colostrum-deprived calf at age 19 days (2), age 40 days (3) and age 67 days (4), and from an individual (1) and from pooled (5) samples of adult bovine γ-globulin.

*Alterations in B chains during neonatal development.* Samples of γ-globulin were isolated from the serum of a colostrum-deprived calf at 19, 40 and 67 days after birth. The isolated B chains from pooled and individual samples of adult bovine γ-globulin contained 11 subfractions; all these could be detected in the B chains at 19 days but the relative concentrations of these bands were different from those of the adult. Further alterations in the B-chain pattern were seen at 40 and 67 days, and faster-moving components became less prominent (Fig. 8).

## DISCUSSION

*Heterogeneity of A chains.* It is apparent that the A chains of 7s γ-globulin are heterogeneous since they carry antibody-combining sites (Fleischman *et al.* 1963) and are associated with the allelic forms of the human *Gm* allotype (S. Lawler, personal communication; see Cohen, 1963*a*); moreover, the chemical heterogeneity of human A chains is indicated by the fact that they contain both aspartic acid and glutamic acid as *N*-terminal groups (Cohen, 1963*c*). In urea–formic acid starch gels, pH 3·5, A chains are relatively homogeneous, but show a broad electrophoretic spread when analysed in urea–glycine starch gels, pH 8. Although attempts to resolve the A chains into distinct electrophoretic components have been unsuccessful, it has been possible to show that the mobilities of A chains are related to those of the original γ-globulins; thus A chains prepared from $\gamma_1$ and $\gamma_2$ subfractions of human 7s γ-globulin have the same relative mobilities as the original proteins.

The A chains of myeloma proteins appear to be identical with normal A chains when analysed in urea–formic acid starch gels (Poulik & Edelman, 1961). However, a study of the genetic characters of human γ-globulins has shown that myeloma proteins contain only one of the alternate *Gm* alleles even in heterozygous individuals (Harboe, Osterland, Mannik & Kunkel, 1962), which indicates that pathological A chains are less complex than normal A chains. In the present study the relative homogeneity of myeloma A chains has been demonstrated in acrylamide gels at pH 8–9; as for normal γ-globulin, mobilities of pathological A chains are related to those of the parent γ-globulins.

*Heterogeneity of B chains.* The heterogeneity of the B chains from γ-globulins of several species is apparent from their electrophoretic behaviour in urea–formic acid starch gels (Edelman & Poulik, 1961; Fleischman *et al.* 1962). It now appears from electrophoresis in urea–glycine starch gel, pH 7–8, that in all species examined the B chains can be resolved into about ten distinct components. It appeared likely that this complexity was an artifact of the method of preparation or fractionation. However, increasing the times of exposure to acid or to isocyanate in alkaline urea or more drastic reduction did not alter the pattern of components observed; in addition, the separated fractions when again subjected to electrophoresis behaved as single components with the same mobilities as those originally observed. The tentative conclusion is therefore drawn that the B chains of γ-globulins exist in the native molecule in a variety of forms, and since these can be separated in gels containing 8M-urea they probably differ in amino acid composition.

This view is supported by the behaviour of the B chains isolated from human pathological γ-globulins. In these proteins the yield of A and B chains is identical with that from normal γ-globulins (Cohen, 1963*c*), indicating that they have the same basic structure, but the B chains consist of only one or two electrophoretic components with mobilities that correspond to one or other of the ten components of normal human B chains. As normal and pathological γ-globulins are isolated and fractionated in exactly the same way, this is the strongest evidence that the multiple nature of the normal B chains is not an artifact. Amino acid analyses were carried out on samples of normal and pathological B chains hydrolysed for 24 hr., and the results corrected for destruction and incomplete hydrolysis from the results obtained in the analysis of whole human B chains (Crumpton & Wilkinson, 1963) (Table 2). These corrections may not be entirely accurate, but it seems clear that, though there are differences between type I B chains of different mobilities, the differences between type I and type II B chains even of the same mobility are much more striking. For example, type I B chains have a significantly higher content of glutamic acid and lower content of proline, glycine, alanine, valine and tryptophan. These findings are in accordance with the observation that the two antigenic types of Bence-Jones proteins, which appear to be composed of B chains (Edelman & Gally, 1962), show completely dissimilar peptide patterns after tryptic digestion (Putnam, Migita & Easley, 1962). Pathological B chains that appear to be identical on electrophoresis in urea–glycine starch gels may differ in antigenic specificity, *N*-terminal amino acid and overall amino acid composition (Tables 1 and 2). This indicates either that myeloma B chains differ from the corresponding subfractions of normal B chains, or that the normal subfractions observed in urea–glycine gels are heterogeneous.

No evidence has been obtained for individual differences in normal B chains, and the patterns observed in African, Chinese and Caucasian subjects

and in the parents of a-γ-globulinaemic children were all identical. This suggests that the complexity of normal B chains does not arise from genetic variability. The relative homogeneity of myeloma B chains supports the idea that different forms of the normal B chains are derived from distinct cell types. Differentiation leading to the synthesis of relatively homogeneous B chains could result from antigenic stimulation. Indeed, Edelman, Benacerraf, Ovary & Poulik (1961) have reported that fully-reduced alkylated guinea-pig antibodies analysed in urea–formic acid starch gels show reproducible patterns of the L (B) fraction that are characteristic for antibodies of different specificities. The authors suggested that this was evidence for chemical differences in the L (B) chains related to antibody specificity. In preliminary experiments it appears that B chains prepared from guinea-pig anti-ovalbumin and anti-(bovine serum albumin) contain all the components of normal B chains when analysed in urea–glycine starch gels, although there were differences in the relative concentrations of the bands (Fig. 6). It appears unlikely, therefore, that the complexity of the B chains can be directly related to antibody specificity. The differences observed between antibody B chains could result from the fact that distinct antigens stimulate different cell populations which synthesize the various forms of B chain in different amounts.

If the complexity of B chains is related to cellular differentiation, alterations in the B-chain pattern might be expected during postnatal development when γ-globulin synthesis increases rapidly. Evidence for this has been obtained by analysis of the B chains isolated from the serum of a colostrum-deprived calf (Fig. 8), but considerably more information will be necessary to establish this apparent relationship. These preliminary findings do, however, suggest that the complexity of the B chains and the occurrence of several enzymes in multiple molecular forms (Levi-Montalcini & Angeletti, 1962) may be related phenomena. The patterns of some of these isoenzymes, including esterases (Markert & Möller, 1959; Markert & Hunter, 1959) and lactate dehydrogenases (Flexner, Flexner, Roberts & de la Haba, 1960; Kaplan, Ciotti, Hamolsky & Bieber, 1960; Fine, Kaplan & Kuftinec, 1963), appear to be tissue-specific and to undergo progressive alteration during pre- and post-natal development.

## SUMMARY

1. The A chains of human γ-globulin are electrophoretically heterogeneous when analysed in urea–acrylamide gels, pH 8·8; the relative mobilities of A chains from both normal and pathological proteins are related to those of the original γ-globulins.

2. In several species the B chains are resolved into multiple electrophoretic subfractions when analysed in urea–glycine starch gels, pH 7–8. The B chains of human 7s and 19s γ-globulin are identical. The observed complexity of B chains cannot be attributed to differences between individuals or to differences between specific antibodies. The distribution of B chains in the γ-globulin of a colostrum-deprived calf altered considerably between 3 and 10 weeks after birth.

3. The B chains from pathological 7s, $\gamma_1$M- or $\gamma_1$A-globulins are relatively homogeneous and consist of one or two components that usually have mobilities corresponding to one or other of the ten subfractions of normal human B chains. Pathological B chains appear to differ in amino acid composition and the differences are most marked between B chains of distinct antigenic types. These findings suggest that the complexity of normal B chains may result from the synthesis of chemically distinct B chains by different cell types.

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# Subcellular Redistribution of Liver α-Glucan Phosphorylase during Alterations in Glycogen Content

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A large fraction of hepatic glycogen is distributed in the cell as 'particulate' glycogen, which is associated with the smooth endoplasmic-reticulum membranes (Lazarow, 1942; Claude, 1954; Porter & Bruni, 1959; Luck, 1961; Drochmans, 1963). It is also known that glycogen exhibits both intramolecular and intermolecular differences of chemical and metabolic labilities (Stetten, Katzen & Stetten, 1956; Figueroa & Pfeiffer, 1962; see Stetten & Stetten, 1960). The possibility of localizing particulate and non-particulate glycogen raises the question whether there exists a special relationship between the subcellular distribution of the enzymes concerned with glycogen metabolism and the structural disposition of the polymer. Luck (1961) conclusively demonstrated that UDP-glucose–glycogen glucosyltransferase [uridine diphosphate glucose–α-(1→4)-glucan α-4-glucosyltransferase, EC 2.4.1.11], a key enzyme in the synthesis of glycogen (see Leloir & Cardini, 1962), was firmly bound to particulate glycogen obtained after a series of cell fractionations. Reliable information, based on similar studies of subcellular distribution, was not available for α-glucan phosphorylase [α-(1→4)-glucan–orthophosphate glucosyltransferase, EC 2.4.1.1], the major enzyme for the degradation of glycogen (see Stetten & Stetten, 1960; Krebs & Fisher, 1962). Madsen & Cori (1958) had, however, studied the interaction between purified corn glycogen and muscle phosphorylase and described the physicochemical features of the binding. Some recent studies also suggest that phosphorylase may be associated to some extent with the glycogen-rich liver-microsomal fraction (Sutherland & Wosilait, 1956; Leloir & Goldemberg, 1960; Nigam, 1962; see de Duve, Wattiaux & Baudhuin, 1962). Particulate glycogen was not isolated, nor were enzyme recoveries or the concentration of liver glycogen taken into account in these studies. My interest in systematically defining the relationship between liver phosphorylase and particulate glycogen arose from some observations made on partial glycogen depletion during the early cellular action of thyroid hormones (Tata *et al.* 1963).

The present paper deals with the subcellular localization of rat-liver phosphorylase under different conditions. It is shown that phosphorylase is normally firmly associated with particulate glycogen, but that the enzyme is reversibly redistributed in the soluble fraction if glycogen is depleted by starvation or the administration of puromycin and 3,3′,5-tri-iodo-L-thyronine. The binding of phosphorylase to glycogen was also studied in artificial systems with preparations of isolated glycogen of different molecular sizes.

## EXPERIMENTAL

*Materials.* Dog-liver glycogen ($S_{20,w}$ = 28·0s), containing less than 0·3% of N, was purchased from Roche Products Ltd., Welwyn, Herts. Rat-liver glycogen of low molecular weight was prepared by the classical KOH extraction followed by precipitation with ethanol (Stetten *et al.* 1956). The dimethyl sulphoxide extraction procedure of Whistler & BeMiller (1962) was adopted for less-degraded or high-molecular-weight preparations of rat-liver glycogen. In such preparations, the main glycogen component had a sedimentation constant, $S_{20,w}$, of 130s, compared with 76s for the alkali–ethanol preparation. Glucose 1-phosphate, glucose 6-phosphate, phosphoenol-