Rabbit Immunoglobulin Lacking Group *a* Allotypic Specificities. I. Isolation and Nature of Heavy Chain†

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ABSTRACT: The group *a* allotypic specificities (*a*1, *a*2, and *a*3) are segregating genetic markers present on the Fd portion of each class-specific heavy (H) chain (γ, μ, α, and ϵ) of rabbit immunoglobulin and have been shown to correlate with the N-terminal primary structure of the γ and α chains. H chains lacking the group *a* specificities also exist. Immunoglobulins containing non-*a* H chains were obtained by suppression of the group *a* markers in homozygous rabbits through embryo transfer and injection of the neonates with antiallotype sera. This paper reports an investigation into the structural relationships between these non-*a* chains and the corresponding *a* chains. Evidence is provided that the Fc fragments from *a* and non-*a* chains are neither serologically nor chemically distinguishable. It seems unlikely that the non-*a* chains are a subclass of normal rabbit chains.

The discovery of allotypy within the rabbit immunoglobulins (Oudin, 1956) made possible studies of the genetic control of their synthesis. Allotypes are specificities on protein molecules which are under genetic control and differ within the species. While they are usually detected serologically, it is believed that allotypic specificities probably correlate with genetically determined variations in the primary structure of the protein. The general subject of rabbit allotypy has been reviewed by Mage *et al.* (1973).

This series of papers will be concerned primarily with the allotypic specificities of group *a*, *i.e.*, the alleles *a*1, *a*2, and *a*3. By serologic criteria these specificities have been identified on the heavy (H) chains[1] from each of the four immunoglobulin classes in the rabbit. They correlate with characteristic compositions and sequences in the variable (V_H) regions of the γ and α chains (Wilkinson, 1969; Mole *et al.*, 1971). This distribution of specificities poses an enigma with respect to the genetic control mechanisms for immunoglobulin synthesis as it appears to predicate that each class of H chains, while retaining a constant individuality in its carboxy-terminal constant (C_H) region, participates in a common genetic polymorphism in its V_H region. The "two gene–one polypeptide chain" hypothesis attempts to reconcile this paradox by postulating that a single H chain requires the interaction of a class-specific C_H region gene with the appropriate V_H region gene encoding the allotypic specificity (Todd, 1972). Two other sets of allotypic specificities will be mentioned in the course of these studies, those of group *b* (*i.e.*, *b*4, *b*5, *b*6, and *b*9), which are found on rabbit κ light (L) chains, and those of groups *d* (*i.e.*, *d*11 and *d*12) and *e* (*i.e.*, *e*14 and *e*15), which are found in the C_H region of γ chains only.

It has been appreciated for some time that some immunoglobulin molecules may lack allotypic specificities from a given group (Oudin, 1961), and through allotype suppression (Dray, 1962) it became possible to expand this population of allotypically deficient immunoglobulins (Dubiski, 1967; David and Todd, 1969). In this paper, data will be presented which demonstrate differences between H chains bearing and lacking group *a* allotypic specificities, but these differences do not appear ascribable to a new subclass of γ chains. Two subsequent publications (Tack *et al.*, 1973; Prahl *et al.*, 1973) present evidence that allotypically blank γ chains are representative of a V_H region subgroup, which may derive from one or more V_H region genes distinct from those encoding the group *a* allotypic specificities.

Materials and Methods

Serologic Methods. Typing of sera for the group *a* and *b* allotypic specificities was done by interfacial precipitation reactions (ring tests). The antiallotype sera were prepared by injection of rabbits with rabbit antiovalbumin specific precipitate (Oudin, 1960). Typing for *d*11 and *d*12 was done by inhibition of hemagglutination (Mandy and Todd, 1970).

Suppression of Group a Allotypes. Rabbits producing immunoglobulins lacking the group *a* allotypic markers were obtained by embryo transfer (David and Todd, 1969) and intraperitoneal injection of the resultant neonates with the appropriate antiallotype serum according to the following schedule: day 1 (birth), 1.25 ml; day 3, 1.25 ml; day 5, 1.25 ml; day 10, 2.5 ml; and day 17, 5.0 ml.

Isolation of IgG. IgG was isolated from pooled rabbit sera by two precipitations with 2.14 M ammonium sulfate followed by chromatography on DEAE-cellulose (Levy and Sober, 1960) equilibrated with 20 mM sodium phosphate buffer (pH 6.6).

Radioiodination of IgG. IgG was tagged with ^{125}I using the method of McConahey and Dixon (1966). To 100 μg of IgG in 1 ml of 0.1 M sodium phosphate buffer (pH 7.4) was added 20 μCi of $Na^{125}I$ (New England Nuclear) and 25 μg of Chloramine-T. The reaction was terminated at 5 min through the addition of 25 μg of sodium metabisulfite. Unbound ^{125}I was removed by exhaustive dialysis against 20 mM sodium phosphate buffer (pH 7.9), 0.15 M in NaCl (PBS).[2] The radio-

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[1] The nomenclature employed for rabbit immunoglobulins, their chains, and fragments follows that recommended by a committee of the World Health Organization (1964).

[2] Abbreviation used is: PBS, 20 mM sodium phosphate (pH 7.9)–0.15 M NaCl.

activity of these preparations varied from 1 to 2 $\times$ 10^8 cpm/mg. The effectiveness of the dialysis step in removing unbound ^{125}I was routinely checked by determining counts precipitable in 10% trichloroacetic acid.

Determination of Radioactivity. Radioactivity was determined in a Nuclear-Chicago crystal scintillation counter equipped with a 512 channel analyzer and a multiple region of interest accessory. ^{125}I was counted from 10 to 70 keV, ^{131}I from 320 to 400 keV, and ^{22}Na from 470 to 550 keV.

Quantification of IgG. A double antibody technique utilizing goat anti-rabbit Fc γ as the primary antibody and horse anti-goat IgG as the secondary (precipitating) antibody was used for the purpose of evaluating the purity of the IgG preparations used in these studies. To a micro test tube was added 10 μl of PBS containing 1 μg of rabbit ^{125}I-labeled IgG, 25,000 cpm (4 μg) of goat ^{131}I-labeled IgG, and 40,000 cpm of ^{22}Na to serve as a volume marker (Gotschlich, 1971). The tube was counted and 50 μl of goat anti-rabbit Fc γ diluted 1:20 in PBS was added. After incubation at 37° for 2 hr, 100 μl of undiluted horse anti-goat IgG was added, and the incubation continued for 1 hr at 37° and 15 min at 4°. The tube was centrifuged (15,000*g*) for 5 min, the supernatant reduced in volume to about 20 μl by aspiration, and the residue counted. The inclusion of the goat ^{131}I-labeled IgG was desirable, since an inability to completely precipitate it would signal an error in the assay. For a thorough discussion of the calculations required to determine the per cent of radiolabeled protein precipitated the reader is referred to Egan *et al.* (1972).

Preparation of Antiallotype Immune Adsorbents. The preparation of immune adsorbents through insolubilization of antisera with ethyl chloroformate was first described by Avrameas and Ternynck (1967). Antiallotype sera were insolubilized following the procedure of Landucci-Tosi *et al.* (1970).

Quantification of Group a and b Allotypic Specificities. A procedure similar to that described by Landucci-Tosi *et al.* (1970) was used for the quantification of allotypy. To a series of micro test tubes was added 10 μl of ^{125}I-labeled IgG (1 μg) and an amount of ^{22}Na to give 40,000 cpm. Each tube was counted and the following additions made: 100 μl of PBS containing 1 mg of bovine serum albumin, 5–100 μl of insolubilized anti-allotype serum, and PBS to a final volume of 210 μl. The contents of each tube were mixed and incubated at room temperature for 3 hr. Constant mixing during this time period was provided by placing the reactants on a culture rotator. Following the incubation period, each tube was centrifuged (15,000*g*) for 5 min, the supernatant reduced in volume to $\sim$20 μl, and the residue counted. The per cent of initial ^{125}I counts precipitated for each addition of antiserum was calculated, and a binding curve was constructed. A quantitative assessment of the IgG molecules bearing a given allotypic specificity could be made, provided the binding curve clearly plateaued. Controls included tubes to which no antiserum was added and tubes with antisera of a group *a* specificity other than that of the test antigen.

Immunoelectrophoresis. Slides of 2% Agar-Noble in 50 mM sodium diethyl barbiturate (pH 8.6) were electrophoresed at approximately 3 V/cm across the slide for 1.5 hr (Scheidegger, 1955).

Isolation of Fc γ Fragments. Fc fragments were obtained through digestion of intact IgG with papain (Worthington Biochemical Corp.) as described by Prahl (1967). Crystallization was effected by dialysis of the papain digest against 5 mM sodium phosphate buffer (pH 7.4).

Reduction of IgG and Isolation of γ Chains. IgG was mildly reduced and alkylated according to the procedure of Prahl *et al.* (1969). H and L chains were separated by gel filtration with Sephadex G-100 in 1 N propionic acid at room temperature (Fleischman *et al.*, 1962).

Cyanogen Bromide Cleavage of γ Chains. The γ chains were cleaved with cyanogen bromide using the method of Givol and Porter (1965). The cyanogen bromide fragments were separated by gel filtration through a column (2.5 $\times$ 180 cm) of Sephadex G-100 in 50 mM sodium formate buffer (pH 3.2), 6 M with urea.

Peptide Mapping of Fc γ Fragments. Fc γ fragments were fully reduced with dithiothreitol, alkylated with [1-^{14}C]iodoacetamide, and digested with trypsin (Small *et al.*, 1966). The chromatographic and electrophoretic systems used to develop peptide maps were those of Katz *et al.* (1959): descending chromatography in 1-butanol–acetic acid–water (4:1:5) followed by electrophoresis in pyridine–acetate buffer (pH 3.5).

Amino Acid Analyses. Amino acid analyses were performed with a Beckman Model 121 amino acid analyzer using 53- and 8-cm columns for the separation of acidic and neutral plus basic amino acids, respectively. Each sample to be analyzed was hydrolyzed for 24 and 72 hr. Maximal values for serine and threonine were obtained through linear extrapolation to zero time.

TABLE I: Antiallotype Sera.

Specificity	Rabbit	Allotype	Immunizing Antigen
Anti-*a*1	1973	*a*2,*b*4,*d*12	*a*1,*b*4,*d*12
Anti-*a*2	2277	*a*1,*b*4,*d*11,*d*12	*a*2,*b*4,*d*12
Anti-*a*2	2278	*a*1,*b*4,*d*11,*d*12	*a*2,*b*4,*d*12
Anti-*a*3	1818	*a*1,*b*4,*d*11,*d*12	*a*3,*b*4,*d*11
Anti-*a*3	1897	*a*1,*b*4,*d*12	*a*3,*b*4,*d*11
Anti-*b*4	1700	*a*1,*b*5,*d*12	*a*1,*b*4,*d*12

Results

The antiallotype sera used in these studies for suppression and quantification of allotypic specificities are shown in Table I.

The origin of immunoglobulins lacking the group *a* allotypic specificities are shown in Table II which indicates for each suppression experiment the number and allotype of the surrogate doe and the number and phenotypic allotype of the offspring. The sera of suppressed rabbits were pooled as indicated and assigned pool numbers I–IV. An impression as to the success of the embryo transfer experiments is provided by considering the first experiment (Table II) in detail. Fourteen fertilized eggs (genotype *a*1,*b*4,*d*12) were implanted into the oviducts of rabbit 3011. Eight young were kindled by 3011. Of these, five were completely suppressed for the *a*1 specificity following postnatal exposure to anti-*a*1 serum.

The IgG from suppressed and normal homozygous rabbits was not serologically distinguishable other than by the absence or presence of the group *a* specificities. Immunoelectrophoreses of *a*1,*b*4, and $a\bar{1}$,*b*4 IgG revealed identically positioned precipitin lines when developed with goat anti-whole rabbit serum (Figure 1; upper panel). However, when developed with anti-*a*1, a single precipitin line was observed for *a*1,*b*4 IgG only (Figure 1, lower panel). Identical results were obtained when $a\bar{2}$,*b*4,*b*5 and $a\bar{3}$,*b*4 IgG were immuno-

TABLE II: Origin of IgG Lacking the Group *a* Allotypic Specificities.

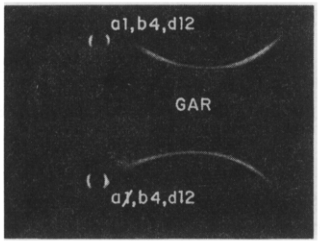
Surrogate Doe	Allotype	Offspring	Allotype[a]	Pool
3011	*a*2,*b*4,*d*12	3423	$a\bar{1}$,*b*4,*d*12	I
		3424		
		3425		
		3426		
		3428		
2752	*a*1,*a*3,*b*4,*b*5,*d*12	3697	$a\bar{2}$,*b*4,*d*12	II
		3700		
		3701		
		3834		
3322	*a*3,*b*5,*d*11	3833	$a\bar{2}$,*b*4,*b*5,d*12*	II
		3836		
		3839		
3062	*a*1,*a*2,*b*4,*d*11,*d*12	3508	$a\bar{3}$,*b*4,*d*12	III
		3511		
3062	*a*1,*a*2,*b*4,*d*11,*d*12	3509	$a\bar{3}$,*b*4,*d*11,*d*12	III
		3510		
3333	*a*1,*a*2,*b*4,*b*5,*d*12	3852	$a\bar{3}$,*b*5,*d*11	IV
		3853		

[a] Phenotypic allotype (*e.g.*, a rabbit of genotype *a*1,*b*4,*d*12 which does not phenotypically express *a*1 is noted $a\bar{1}$,*b*4,*d*12).

electrophoresed along with their corresponding controls. Ouchterlony gel diffusion analysis with goat anti-rabbit Fc γ indicated complete serologic identity for both the *a* and the non-*a* IgG's (Figure 2).

A quantitative assessment of the allotypic specificities present on *a* and non-*a* IgG was possible by radioimmune assay using anti-*a* and anti-*b* allotype sera which had been insolubilized with ethyl chloroformate. The binding profiles of *a*1,*b*4 and $a\bar{1}$,*b*4 IgG, obtained with anti-*a*1 and anti-*b*4, are shown in Figure 3. Since the binding curves clearly plateaued, a quantitative determination of each specificity was possible. The *a*1,*b*4 IgG was 95% precipitable with anti-*a*1 and 94% with anti-*b*4. The $a\bar{1}$,*b*4 IgG, however, lacked the *a*1 specificity but retained complete *b*4 specificity (94%). IgG from rabbits suppressed for specificity *a*2 similarly lacked its respective *a* markers and retained *b* markers (pool II, Table III). When tested with anti-*a*3 antisera, the IgG from rabbits suppressed for the *a*3 specificity gave negative interfaced precipitation reactions. With insolubilized anti-*a*3, however, considerable precipitation of these same IgG pools was found (pools III and IV, Table III). The observations leading to the conclusion that these anomalous results do not represent *a*3 specificity is presented in the following section. Irrespective of the group *a* allotype, the fact that precipitability of IgG from young nonimmunized rabbits never exceeded 95% suggests that non-*a* IgG is normally present, but in limited amounts. The *a*2,*b*4 IgG was particularly striking in that anti-*a*2 precipitated only 80% of the molecules. The precipitabilities of the IgG preparations were 97.1–99.2% with goat antirabbit Fc γ (Table III).

The amino acid compositions of the γ chains isolated from the IgG of normal control and suppressed rabbits are given in Table IV. These data may be evaluated in three ways; *i.e.* by comparison of *a*1, *a*2, and *a*3 γ chains with each other, by comparison of $a\bar{1}$, $a\bar{2}$, and $a\bar{3}$ γ chains with each other, and finally by comparison of each non-*a* γ chain with its normal counterpart. Koshland *et al.* (1968) have previously reported



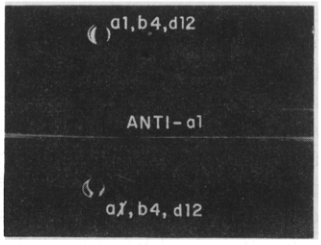


FIGURE 1: Upper panel: immunoelectrophoretic comparison of *a*1,*b*4 and $a\bar{1}$,*b*4 IgG developed with goat anti-whole rabbit serum (GAR) ($\bar{1}$ in the text is represented in the figures by the number with a slant). Lower panel: immunoelectrophoresis of *a*1,*b*4 and $a\bar{1}$,*b*4 IgG developed with anti-*a*1. The concentration of each antigen was approximately 20 mg/ml in 20 mM sodium phosphate buffer (pH 6.6).

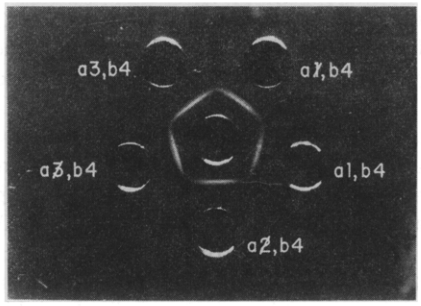


FIGURE 2: Ouchterlony gel diffusion reactions of IgG from normal and suppressed rabbits (peripheral wells) with goat anti-rabbit Fc γ (center well). The concentration of each antigen was 1.5 mg/ml in 20 mM sodium phosphate buffer (pH 6.6).

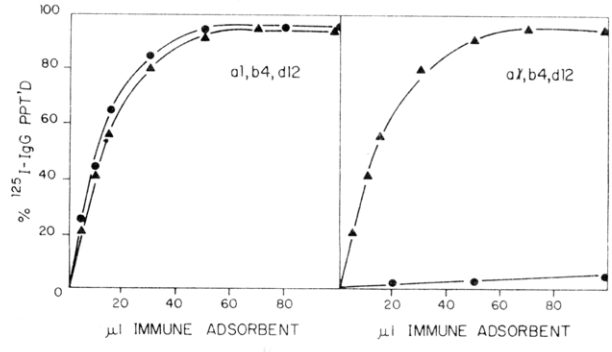


FIGURE 3: Binding curves generated by the addition of increasing volumes of insolubilized anti-*a*1 (●) and anti-*b*4 (▲) to ^{125}I-labeled *a*1,*b*4 (left panel) and $a\bar{1}$,*b*4 (right panel) IgG ($\bar{1}$ is represented in the figure by the number with a slant). Each point represents an average of at least two determinations.

TABLE III: Quantification of Allotypes Present on IgG.[a]

Pool	Allotype	% Precipitated					
		Anti-*a*1	Anti-*a*2	Anti-*a*3[b]	Anti-*b*4	Anti-*b*5	Anti-Fc γ
I	$a\bar{1}$,*b*4,*d*12	4.0			97.0		99.0
II	*a*2,*b*4,*b*5,*d*12		5.0		75.0	15.0	99.2
III	$a\bar{3}$,*b*4,*d*11,*d*12			20.0	94.7		97.1
IV	$a\bar{3}$,*b*5,*d*11			45.6		91.5	99.2
V[c]	*a*1,*b*4,*d*12	94.9			95.6		98.4
VI[c]	*a*2,*b*4,*d*12		80.0		85.2		98.2
VII[c]	*a*3,*b*4,*d*12			94.8	87.3		97.6

[a] The data have not been corrected for a background precipitation level of 1–2%. [b] The unexpected precipitation of pools III and IV with anti-*a*3 is treated in the Discussion. [c] These are IgG's from unsuppressed rabbits of the same age as the suppressed rabbits and have been used as normal controls.

variations in amino acid composition which appeared associated with the γ-chain group *a* allotype. Although the degree of variability was not always the same as that seen by Koshland and coworkers (1968), differences in alanine, arginine, aspartic acid, isoleucine, phenylalanine, proline, threonine, and valine have also been observed in the present study. In addition variations in glutamic acid, serine, and tyrosine have been observed.

Of more relevance to the present study, however, was the loss of this pattern of allotype associated variation when a comparison was made among the compositions of $a\bar{1}$, $a\bar{2}$, and $a\bar{3}$ γ chains. Although variations are seen among the non-*a* chains the pattern and degree of variability are quite different from that seen among *a*-bearing chains, and its significance is uncertain at this time. Finally, in all cases the threonine content of non-*a* chains was markedly less than that of *a*-bearing chains. Other minor compositional variance was also observed between non-*a* γ chains and the chains bearing the respective allotypes.

TABLE IV: Amino Acid Composition of H Chains.[a]

Amino Acid	H-Chain Allotype					
	*a*1,*d*12	$a\bar{1}$,*d*12	*a*2,*d*12	$a\bar{2}$,*d*12	*a*3,*d*12	$a\bar{3}$,*d*11
Ala	21.7	22.4	23.6	22.2	24.9	22.9
Arg	17.2	17.3	16.9	16.8	15.5	16.4
Asp	31.5	32.0	34.2	31.8	31.4	33.0
Cys-CH_2COOH	5.0	5.3	4.8	4.2	3.8	5.0
Cys	9.9	8.4	9.7	10.3	9.9	9.3
Glu	37.1	39.4	37.0	38.7	38.8	37.9
Gly	32.3	34.3	32.1	33.4	32.3	32.5
His	5.9	6.1	5.8	6.1	5.9	6.3
Ile	14.7	14.4	14.9	14.2	13.8	14.7
Leu	30.9	31.3	30.7	30.8	30.9	31.6
Lys	22.6	21.9	22.4	22.8	22.6	22.8
Met	4.8	4.8	5.1	4.7	5.2	5.9
Phe	14.6	14.4	14.8	14.7	15.4	14.4
Pro	38.7	39.3	37.5	39.0	37.9	40.2
Ser	49.9	52.4	48.9	52.9	50.5	51.7
Thr	48.6	41.8	47.5	42.6	47.2	41.0
Tyr	15.7	16.2	15.0	15.7	16.0	16.2
Val	39.5	38.6	39.0	39.0	38.1	38.9

[a] Compositions are reported as amino acid residues per 440 residues. Cysteine and methionine were determined as cysteic acid and methionine sulfone, respectively (Moore, 1963).

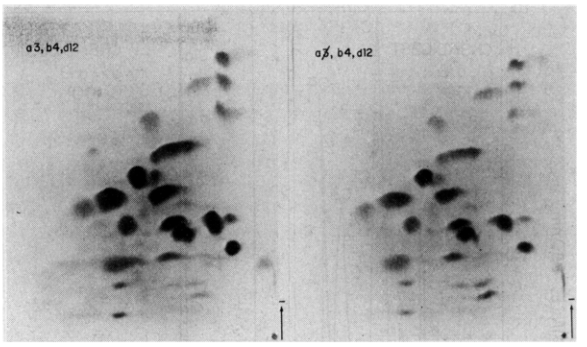


FIGURE 4: Fingerprints of trypsin digested Fc γ fragments stained with ninhydrin. Left panel: the peptide map of Fc γ from the *a*3,*d*12 H chain. Right panel: the peptide map of Fc γ from the $a\bar{3}$,*d*12 H chain ($\bar{3}$ is shown in the figure by the number with a slant). Descending chromatography was in 1-butanol–acetic acid–water (4:1:5) and electrophoresis in pyridine–acetate buffer (pH 3.5).

Peptide maps of the Fc γ fragments from *a*3,*b*4,*d*12 and $a\bar{3}$,*b*4,*d*12 IgG's reduced and alkylated with [1-^{14}C]iodoacetamide did not differ. Figure 4 shows the peptides as stained with ninhydrin. Similarly, the radioautographs were indistinguishable. By either of the above criteria, the Fc γ fragments of $a\bar{1}$,*b*4,*d*12 and $a\bar{2}$,*b*4,*b*5,*d*12 IgG's were not distinguishable from one another or from their respective controls.

Cyanogen bromide cleavage of normal rabbit chains yields four characteristic peptides (C1, C3, C4, and C5) as indicated in Figure 5; an additional peptide (C1c) is derived from chains which carry the *d*11 determinant (Prahl *et al.*, 1969). The elution patterns of these peptides from filtration of CNBr digests of *a*1,*d*12 and $a\bar{1}$,*d*12 γ chains through Sephadex G-100 are shown in Figure 6. Identical gel filtration patterns were observed for the CNBr digests of $a\bar{2}$,*d*12 and $a\bar{3}$,*d*11,*d*12 γ chains.

Fractions from peak C5 (C-terminal octadecapeptide) of each profile were separately pooled, freed of urea by passage through Sephadex G-25 in 50 mM NH_4OH, and lyophilized. The C5 peptide from each digest was further purified by gel filtration through a 1.5 × 240 cm column of Sephadex G-50 equilibrated with 50 mM NH_4OH. The amino acid composition of the C5 peptide is invariant, whether derived from an *a* or a non-*a* γ chain. The compositions of C5 from *a*3,*d*12 and $a\bar{3}$,*d*12 γ chains are shown in Table V.

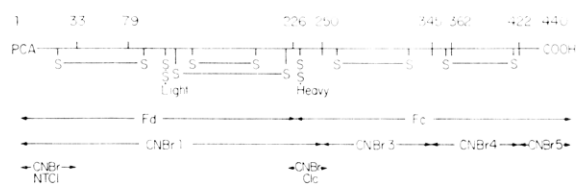


FIGURE 5: Structure of rabbit γ chain showing the positions of the methionine residues. Constant (integral) methionine residues are indicated by a full vertical bar. Occasional (fractional) or allotype-related methionine residues are indicated by a half-bar. The numbering shown is based on a chain of 440 residues in length. In addition, the interchain and intrachain disulfides are shown based on O'Donnell *et al.* (1970). The nomenclature of the fragments of the γ chain obtained by papain or cyanogen bromide cleavage is indicated.

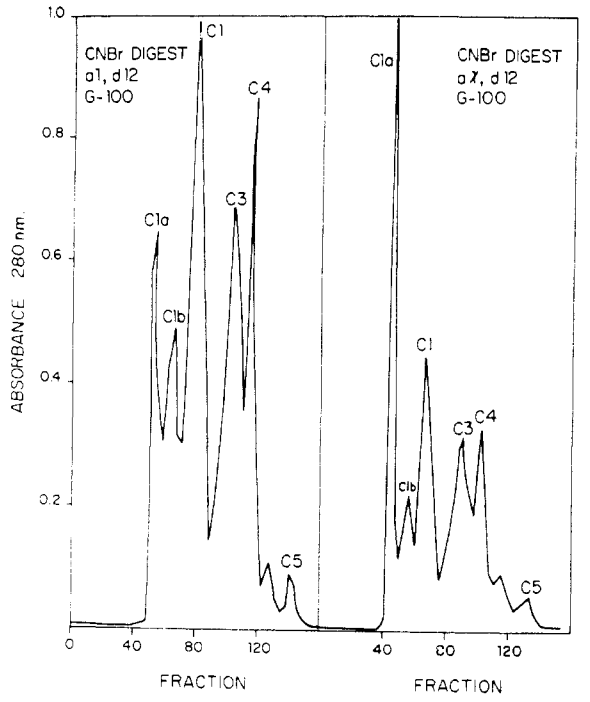


FIGURE 6: Gel filtration of cyanogen bromide digests through a 180 × 2.5 cm column of Sephadex G-100 equilibrated with 50 mM sodium formate buffer (pH 3.2), 6 M with urea. Left panel: CNBr profile of 240 mg of the $a1,d12$ γ chain. Right panel: CNBr profile of 90 mg of the $a\bar{1},d12$ γ chain.

TABLE V: Amino Acid Composition of Fc γ[a] and C5[b] Peptides.

	Fc γ		C5	
Amino Acid	$a3,d12$	$a\bar{3},d12$	$a3,d12$	$a\bar{3},d12$
Ala	8.6	8.3	1.00	1.11
Arg	11.1	10.8	1.07	0.98
Asp	19.0	18.6	1.00	1.19
Cysteic acid	4.1	3.9		
Glu	25.6	25.2	0.97	1.09
Gly	11.0	10.8	1.17	1.12
His	5.1	4.7	2.93	2.59
Ile	10.1	10.2	0.97	1.07
Leu	14.8	15.3	1.00	1.11
Lys	14.6	13.9	0.97	1.09
Met	4.3	3.9		
Phe	8.4	8.8		
Pro	24.0	23.3	0.97	1.09
Ser	18.5	21.3	2.84	2.92
Thr	14.7	14.8	0.97	1.08
Tyr	8.0	8.3	0.92	0.41
Val	18.3	17.8		
Total	220.0	220.0	18.00	18.00

[a] Calculated on the basis of 220 residues. [b] Calculated on the basis of 18 residues.

Discussion

Modulation of the phenotypic expression of allotype genes in young rabbits through *in vivo* exposure to antiallotype sera was observed by Dray (1962). Immunoglobulins from young $b4/b5$ rabbits born of a $b4/b4$ mother immunized against the $b5$ allotype of their father ($b5/b5$) were found to lack the paternal $b5$ allotype. The loss of the $b5$ allotype was compensated by an increase in the level of the maternal $b4$ allotype. Suppression of the paternal allotype was thought to be a manifestation of *in utero* transfer of anti-$b5$ antibody. Subsequent experiments (Mage and Dray, 1965) were in accord with this theory as neonatal injection of antiallotype sera similarly effected the phenotypic suppression of the paternal $b5$ allotype. Dubiski (1967) reported the suppression of the $b5$ allotype in homozygous rabbits through neonatal injection of the young from a $b4/b5$ doe suppressed for $b5$ and bred to a $b5/b5$ buck. An increased level of immunoglobulin lacking the $b5$ allotype was present in the serum of the young $b5/b5$ rabbits. By definition then, suppression of only one allele in individuals heterozygous at a locus is known as heterozygous suppression, whereas homozygous suppression pertains to that situation in which the individual is homozygous at the locus in discussion.

In the present studies, homozygous suppression was achieved by embryo transfer and injection of the neonates with antiallotype sera (David and Todd, 1969). Rabbits suppressed for the group a allotypes ($a1$, $a2$, and $a3$) were weaned at 8 weeks and bled at weekly intervals thereafter. Irrespective of the allotypic genotype of the suppressed rabbit, immunoglobulin bearing the a marker became detectable serologically by the fifth month after birth. Heterozygous suppression, however, may persist for as long as 3 years in the rabbit (Mage and Dray, 1965). Since the mechanism of suppression is not known, this apparent facile release from homozygous suppression is not understood. The transient nature of suppression required that the sera of littermates be pooled in order to have sufficient material for structural studies.

Immunoglobulins lacking the group a allotypes were not serologically distinguishable from the IgG of control rabbits either by immunoelectrophoresis or gel diffusion when developed with goat anti-whole rabbit serum or with goat anti-rabbit Fc γ. By radioimmune assay non-$a1$ and non-$a2$ IgG preparations were <5% precipitable with anti-$a1$ and anti-$a2$, respectively, but >99% precipitable with goat anti-rabbit Fc γ and >97% precipitable with antiallotype sera directed against their respective light-chain specificities. The low precipitability of $a2,b4,d12$ IgG with anti-$a2$ (80%) and anti-$b4$ (85%) was suggestive of an unusually high non-a H and non-b L (λ) chain content since the precipitation with goat anti-rabbit Fc γ remained high (98%).

Only those weekly bleedings of littermates which did not type by ring test for the suppressed $a3$ allotype were pooled. The $a3$ specificity at the 5% level can be detected by the interfacial precipitin test with confidence. Surprisingly, the IgG from serum bleedings (pools III and IV) which did not type for the $a3$sp ecificity by ring test typed as high as 20 and 46%, respectively, by direct binding radioimmune assay irrespective of the anti-$a3$ antisera used (1818 or 1897). The significance of these results is unclear. The possibility that a constant region marker, *i.e.* $e14$ or $e15$ (Dubiski, 1969; Landucci-Tosi *et al.*, 1970), was responsible for the unexpected precipitation was investigated. Both anti-$a3$ antisera were kindly typed by S. Dubiski (personal communication) for the presence of anti-$e14$ or anti-$e15$, and neither was present. The remote possibility that the presence of the $d11$ specificity was responsible for the precipitation with antiserum 1897 was checked by attempts to inhibit precipitation in the presence of a one- to fourfold excess of either unlabeled $a1,b4,d12$ or $a1,b4,d11$ IgG. The precipitation of pools III and IV was unaffected by the presence of the $d11$ specificity. Further, the precipitation of $a3,b4,d12$ IgG labeled with ^{125}I with anti-$a3$ was unaffected

by the presence of unlabeled IgG from pools III and IV. Sufficient amounts of IgG from pools III and IV were added such that the presence of the *a*3 specificity at the levels indicated by the direct binding radioimmune assay would have registered inhibitions of 33 and 52%, respectively. Presently we do not understand this phenomenon, but we do not believe that it is due to the *a*3 specificity.

As well as lacking the serological determinant for a group *a* specificity, the non-*a* γ chains were found to differ from the *a*-bearing chains in amino acid composition (Table IV). Indeed, the non-*a* chains no longer demonstrated the pattern of compositional variations previously associated with group *a* allotypy (Koshland *et al.*, 1968). However, the Fc γ fragments prepared from *a* and non-*a* IgG's were indistinguishable on the basis of amino acid composition or peptide maps of tryptic digests (Figure 4 and Table V). This same observation had been made previously on the Fc γ fragments of *a*1, *a*2, and *a*3 IgG (Small *et al.*, 1966). Prahl and Todd (1971) and Knight *et al.* (1971) have reported that the Fc γ fragments from *a*2 and $a\bar{2}$ IgG were indistinguishable by peptide mapping. The identicality of the carboxyl-terminal halves of *a* and non-*a* γ chains is further supported by the similar pattern of fragmentation obtained upon CNBr cleavage (Figure 6) and the similar amino acid composition of the C-terminal octadecapeptide from each chain (Table V). Minor variations in primary structure, however, cannot be excluded short of complete sequence studies.

In all species investigated to date subclasses of IgG have been characterized by significant structural variation in the Fc γ fragment. This does not appear to be the situation here, despite the variation observed upon comparison of the amino acid composition of *a* and non-*a* γ chains. It seems unlikely that suppression of the group *a* allotypes can be explained by the appearance of a new IgG subclass of rabbit immunoglobulin.

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