PRESENCE OF TWO TYPES OF L POLYPEPTIDE CHAINS IN GUINEA PIG 7S IMMUNOGLOBULINS*

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Previous studies on the structure of guinea pig 7S immunoglobulins (γ_1 and γ_2) (1, 2) have shown that, similar to other mammalian immunoglobulins (reviewed in references 3 and 4), they are formed by two pairs of dissimilar polypeptide chains (H and L), which can be separated by mild reduction and subsequent filtration through a Sephadex column equilibrated with denaturing agents. These immunoglobulins differ mainly through part of their H chain, the Fc fragment (5). It is the purpose of this and a companion paper to present evidence for the presence within both classes of guinea pig 7S immunoglobulins of two different types of L chains (κ and λ)[1] which have distinct antigenic determinants and similar molecular weights.

Materials and Methods

Proteins.—Normal guinea pig γ_2-globulin was prepared from serum by combined ammonium sulfate precipitation and DEAE-cellulose chromatography as described in reference 5. A commercial preparation of guinea pig γ-globulins obtained from Pentex, Inc., Kankakee, Illinois, was also used; it contained at least 95% of γ_2-globulin as verified by precipitation of an I^{131}-labeled sample by specific antisera. This preparation was also further purified by chromatography on DEAE-cellulose. Bovine gamma globulin (BGG) and bovine fibrinogen (BF) were obtained from the Armour Pharmaceutical Co., Kankakee, Illinois.

Preparations of Antigens and Antibodies.—Protein conjugates were prepared by reaction of 2,4-dinitrofluorobenzene (DNP) (6) or *p*-iodobenzenesulfonylchloride (pipsyl) with BGG or BF under alkaline conditions, and subsequent dialysis. Protein concentrations were determined by Kjeldahl analysis and the approximate degree of DNP substitution was calculated from the absorbancy of the dinitrophenylated protein at 360 mμ (6). Only highly con-

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[1] This designation, originally introduced for the two types of human L chains, has been chosen because of certain similarities between human and guinea pig types of L chains, but the homology between them has not been definitely established. Whenever possible, a similar nomenclature to that suggested by the World Health Organization Committee on Nomenclature of Human Immunoglobulins has been used (*Bull. World Health Organ.*, 1964, **30**, 447). Immunoglobulin molecules bearing κ- or λ-types of L chains have been called respectively K molecules and L molecules.

jugated DNP_{65}-BGG was used for immunization. The subscript refers to the average number of DNP groups per molecule.

The animals were injected in the foot-pads with 1 mg of the antigen (DNP_{65}-BGG or pipsyl-BGG) in complete Freund's adjuvant (Difco Laboratories, Inc., Detroit). 3 wk later the animals received intradermally 0.4 of the same antigen in four sites and were bled 28 days after the beginning of the immunization.

Anti-DNP antibodies were purified by precipitation of the antibody at equivalence with DNP-BF in the presence of 0.01 M Na_2-ethylenediamine tetraacetate, followed by elution of the washed precipitate with 0.1 M 2,4-dinitrophenol (DNP-OH) in the presence of streptomycin (6).

Anti-pipsyl antibodies were purified in a similar way, using pipsyl-BF as a precipitating antigen and pipsyl-ε-aminocaproic acid (2×10^{-3}M) as the eluting hapten.

H and L Chains.—H and L chains were prepared from normal guinea pig γ_2-globulin and from purified anti-DNP antibodies according to the method described in detail in reference 2, which consists essentially of filtration of mildly reduced protein through Sephadex G-200 in 4 M guanidine-HCl.

I^{131} Labeling.—Either of two methods was used to label γ-globulin and antibody preparations with I^{131} (7, 8). The reactions were carried out in the cold. (*a*) To 1 to 2 mg protein samples dissolved in a small volume of cold phosphate-buffered saline at pH 7.4 was added 5 to 10 μc of carrier-free NaI^{131}. 0.02 mg of chloramine T was then added followed 2 min later by 0.01 mg of sodium bisulfite. (*b*) To 1 to 2 mg protein samples in a carbonate buffer, pH 10, was added 0.1 ml of a solution containing 5 to 10 μc of NaI^{131} in a mixture containing 0.025 ml of a solution of I_2 in KI (I_2 1.23 and KI 1.57 mg/ml).

The labeled proteins were then dialyzed in the cold against several changes of phosphate-buffered saline, pH 7.4.

Geon Block Electrophoresis.—Geon block electrophoresis of guinea pig serum was performed according to the method described in reference 9 and the fractions eluted were lyophilized.

Specific Precipitation.—Specific precipitation reactions using I^{131}-labeled proteins were carried out in the antibody excess zone. An aliquot of the labeled protein was brought to 0.2 ml with saline and 0.3 ml of the antiserum added. After an incubation of ½ hr at 37°C and 72 hr in the cold, the precipitates were washed three times in cold saline and then dissolved and brought to the original volume with 0.1 N NaOH. The percentage of labeled protein precipitated was calculated by comparing the total number of counts in the tube before washing the precipitate with the number of counts in the washed precipitate after it had been dissolved in NaOH. All measurements were performed in a well type counter.

Gel Diffusion Methods.—Double diffusion analysis in Ouchterlony plates (10) was performed in 1% agar in 0.05 M barbital buffer, pH 8.6. Immunoelectrophoresis was carried out as previously described (11).

Rabbit Antisera.—Antisera against L chains (Nos. 1 and 2) were obtained by immunizing rabbits with pure guinea pig L chains obtained from DEAE-purified γ_2-globulin. The animals received a total of 3 mg of L chains incorporated in complete Freund's adjuvant in the foot-pads in 2 successive wk. Then they received 0.5 mg of the same preparation intradermally once a week for 3 wk, and were bled several times beginning 7 days after the last antigen injection. All bleedings contained antibodies reacting with both types of guinea pig L chains (κ and λ). These sera also reacted slightly with the Fc fragment of γ_2-globulin.

Other antisera, containing a high level of antibodies against both H and L chains, were obtained in the following manner. Rabbits (Nos. 5 and 6) were injected with L chains (κ) prepared from purified anti-DNP_{65}-BGG antibodies. 0.3 mg of L chains incorporated in complete Freund's adjuvant was given in the foot-pads. In each of the 4 following wk the rabbits were boosted with 0.15 mg of the same preparation of L chains. In the 6th wk after

the beginning of immunization the animals received 3.5 mg of intact purified anti-DNP antibodies, containing both γ_1- and γ_2-immunoglobulins, incorporated in complete Freund's adjuvant, and were bled 21 days later. Their sera contained antibodies against the major type of L chains (κ) and against both γ_1- and γ_2-H chains.

RESULTS

Studies by Gel Diffusion Methods in Agar.—The immunoelectrophoretic analysis of guinea pig serum, purified γ_2-globulin and purified L chains with

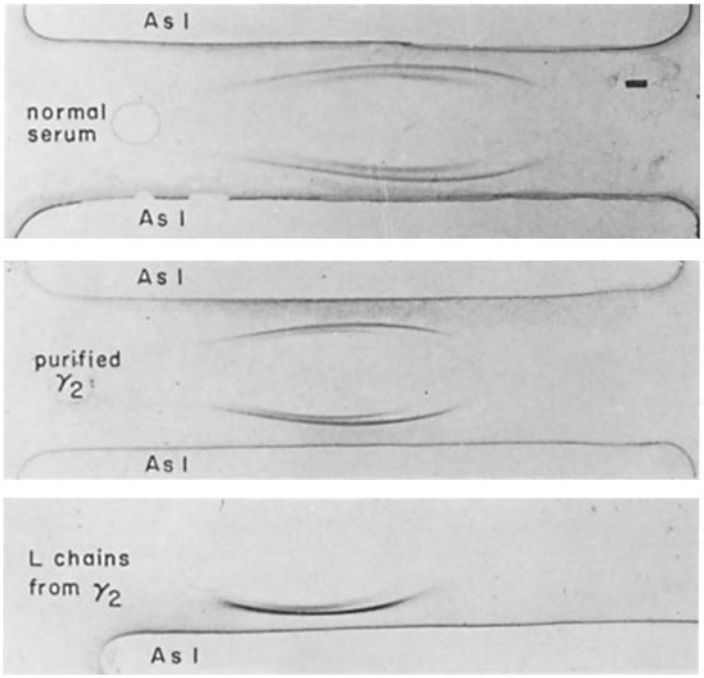

FIG. 1. Immunoelectrophoretic analysis of normal guinea pig serum, purified γ_2-globulin, and L chains isolated from guinea pig γ_2-globulin developed by rabbit antiserum, As 1. Note the two lines corresponding to the two types of guinea pig L chains, κ and λ, and to the two types of γ_2-globulin molecules, K and L.

antiserum 1, prepared against normal guinea pig L chains, shows in every case two precipitin lines corresponding to two distinct components having perhaps a small difference in electrophoretic mobility (Fig. 1). This antiserum distinguishes, therefore, two distinct types of L chains and reacts with two separate γ_2-globulin molecules. However, when purified anti-DNP_{65}-BGG antibody preparations or L chains (κ) prepared from them were analyzed by the same method, only one of these two components was present (Figs. 2 and 3). If antiserum 1 was absorbed with purified anti-DNP_{65}-BGG antibodies as well as

with L chains obtained from them, a specific reagent against only the minor type of L chain was obtained. This antiserum (Figs. 3 and 4) detects only one component in intact guinea pig γ_2-globulins or in the L chains prepared from them. It also shows a reaction of identity when γ_2-globulin and normal L chains are compared by double diffusion analysis (Fig. 5), but does not precipitate guinea pig H chains either in liquid or gelified media (2). These results demonstrate the presence of two types of L chains (κ and λ) in guinea pig γ_2-globulin but only one type of L chain (κ) in purified anti-DNP_{65}-BGG antibodies. The

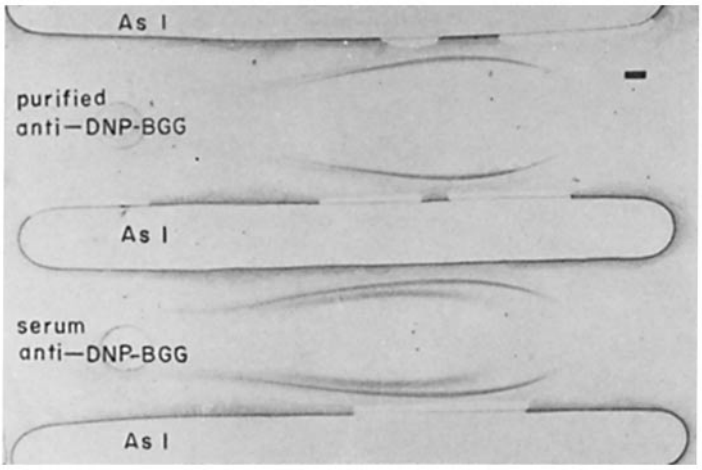

FIG. 2. Immunoelectrophoretic analysis of serum from a guinea pig immunized with DNP_{65}-BGG, and of the anti-DNP antibodies specifically isolated from that serum, developed by rabbit antiserum As 1. Note the two lines corresponding to K and L molecules in the serum pattern and only a single line corresponding to K molecules in the anti-DNP antibody pattern.

reagent obtained by absorption of the immune rabbit sera with guinea pig anti-DNP_{65}-BGG antibodies and their L chains (type κ) is directed only against λ-chains. However, if the absorption of the immune sera is performed only with purified anti-DNP_{65}-BGG antibodies, the resulting reagent still reacts with free κ-chains. It does not precipitate, however, κ-chains present in the intact molecule. In other words, this absorbed serum contains antibodies directed against L chain determinants which are "hidden" or "inactive" when the L chains are combined with the H chains. The presence of these "hidden" determinants in L chains is clearly demonstrated by the immunoelectrophoretic analysis of L chains obtained from γ_2-globulins, which contain both κ- and λ-chains (Fig. 3). When the antiserum absorbed with both κ-chain and anti-DNP_{65}-BGG antibodies is used, only the λ-chains are precipitated, whereas when the antiserum absorbed only with anti-DNP_{65}-BGG is employed, both

κ- and λ-chains are precipitated. Both absorbed antisera, nevertheless, precipitate only the λ-chain–bearing molecules (L molecules) when allowed to react with native γ_2-globulin (Fig. 4).

The presence of both κ- and λ-types of L chain can also be demonstrated in γ_1-immunoglobulins. As pointed out before (12), normal guinea pig serum con-

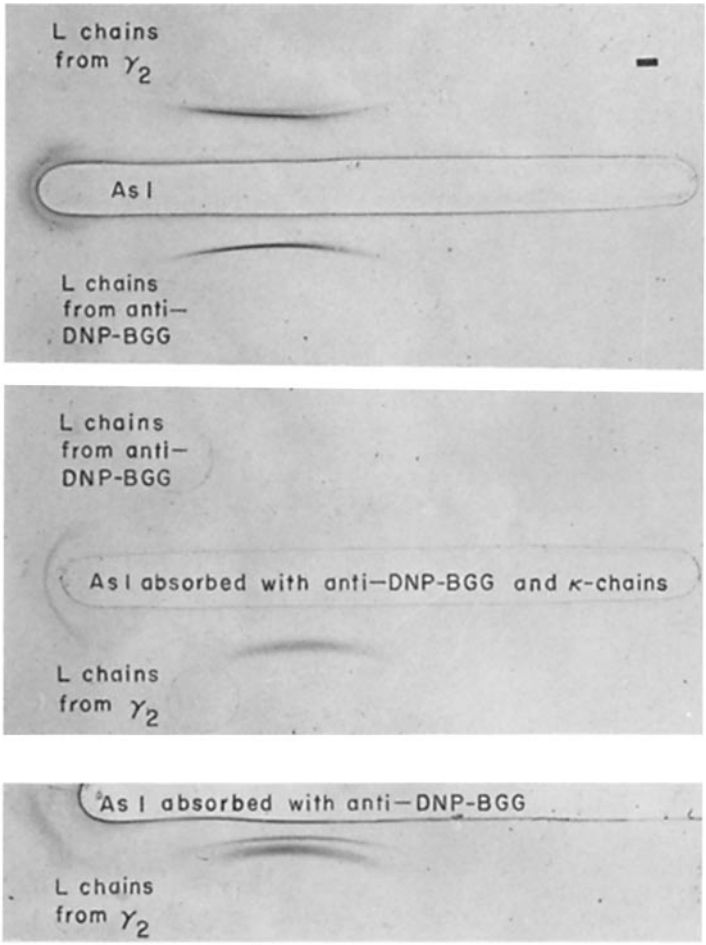

FIG. 3. Immunoelectrophoretic analysis of L chains from guinea pig γ_2-globulin and from guinea pig anti-DNP antibodies isolated from anti-DNP_{65}-BGG serum developed by: (*a*) rabbit serum, As 1; (*b*) the same rabbit antiserum absorbed with anti-DNP antibodies (K molecules) and rendered specific for L molecules; and (*c*) the absorbed rabbit antiserum further absorbed with free L chains from anti-DNP-BGG antibodies (κ-chains) and made specific for λ-chains. Before this last absorption the serum reacted with hidden determinants on free κ-chains but not with κ-chains in K molecules.

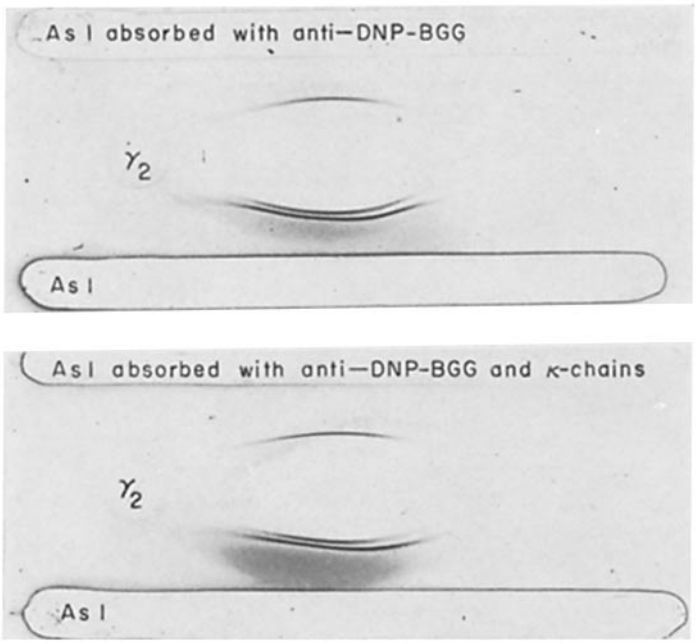

FIG. 4. Immunoelectrophoretic patterns of guinea pig γ_2-globulins developed with a rabbit antiserum (As 1); (*a*) absorbed with anti-DNP-BGG antibodies, and (*b*) absorbed with DNP-BGG antibodies and free κ-chains isolated from these antibodies. Note that the patterns obtained with the absorbed antisera are identical and reveal only the L molecules.

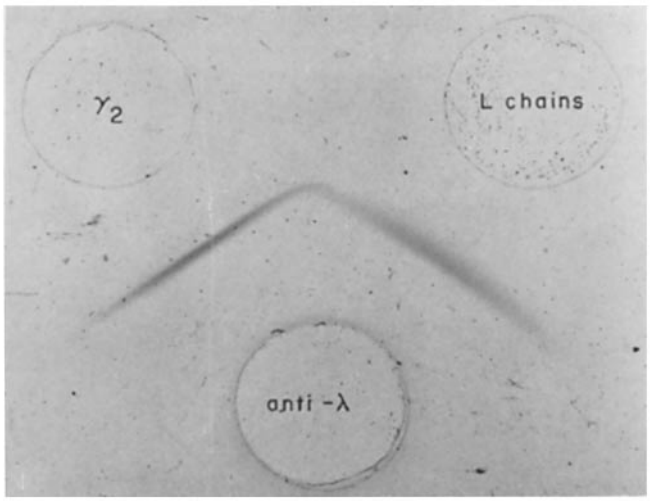

FIG. 5. Double diffusion analysis in agar gel of guinea pig γ_2-globulin and of L chains isolated from guinea pig γ_2-globulin, with rabbit antiserum, As 1, prepared against guinea pig L chains and rendered specific for λ-chains by absorption with guinea pig anti-DNP-BGG antibodies and guinea pig κ-chains. Note that this anti-λ-chain antiserum does not detect any difference between L molecules in γ_2-globulin and λ-chains.

tains very small amounts of this protein, but its concentration may increase greatly in serum during immunization. Purified antihapten antibody preparations may contain considerable amounts of γ_1-immunoglobulin. In such preparations it is possible to demonstrate the presence of both κ- and λ-types of L chains (13). The relative amounts of K and L molecules in the γ_1-fraction parallel remarkably those present in the γ_2-fraction obtained from the same antibody preparation (13).

Quantitative Precipitation Studies with Anti-λ-Antisera.—Since the results presented above demonstrated the existence of two types of L chains in guinea pig immunoglobulins, quantitative precipitation studies were undertaken to evaluate the amount of each type of molecule in the normal γ_2-globulin fraction.

TABLE I

Precipitation of Increasing Amounts of I^{131}-Labeled Guinea Pig γ_2-Globulin by a Constant Volume of a Rabbit Antiserum Specific for the λ-Type of Guinea Pig L Chain*

I^{131}-γ_2 (mg per ml of antiserum)	Labeled protein precipitated	λ-chain in supernatant	I^{131}-γ_2 (mg)precipitated per ml antiserum
	%		
0.260	23.8	−	0.062
0.520	22.7	−	0.118
0.750	23.8	−	0.178
0.820	24.5	−	0.200
1.640	19.3	±	0.316
2.460	11.4	+	0.280
3.280	7.3	+	0.239
4.920	1.5	+	0.074

* DEAE-purified fraction II (Pentex).

These studies were performed by labeling the antigen with I^{131} and then precipitating it in the antibody excess zone with specific anti-λ-antiserum. The antibody excess zone was determined by quantitative precipitation experiments as shown in Table I. Increasing amounts of I^{131}-labeled γ_2-globulin were precipitated by a constant volume of rabbit anti-λ-antiserum. In the antigen excess zone the percentage of antigen precipitated declined sharply, but, as expected, this percentage was fairly constant throughout the antibody excess zone. The rabbit 1 serum absorbed with purified guinea pig anti-DNP_{65}-BGG antibodies was used as a specific anti-λ-chain reagent. As mentioned before this antiserum also contains antibodies directed against "hidden" determinants of κ-chains, but, as shown in Table II and Fig. 4, these antibodies do not react with κ-chains when these are combined with H chains in native γ-globulin molecules.

In most of the studies to be presented only one anti-λ-chain antiserum was used (No. 1), but in a few instances the serum 2 was also employed. As shown

in Table III both antisera precipitate approximately the same amount of labeled protein from different antigen preparations. Using these antisera it could be determined that approximately one-third of normal guinea pig γ_2-

TABLE II

Precipitation of I^{131}-Labeled γ_2-Globulin and Purified Guinea Pig Antibody Preparations by a Rabbit Antiserum Prepared against Guinea Pig L Chains, and Rendered Specific for the λ-Type by Absorption*

Rabbit antisera	Per cent precipitated of		
	Normal γ_2*	Anti-pipsyl-BGG 4	Anti-pipsyl-BGG 1
Rabbit 1, absorbed with anti-DNP antibodies	25.1	23.3	53.8
Rabbit 1, absorbed with anti-DNP antibodies and κ-chains	23.9	23.2	53.2
Normal rabbit serum	0.9	0.8	0.8

* DEAE-purified fraction II (Pentex).

TABLE III

Precipitation of Guinea Pig γ_2-Globulin and of Purified Antibody Preparations by Two Different Rabbit Antisera Prepared Against Guinea Pig L Chains and Rendered Specific for the λ-Chain by Absorption with Purified Anti-DNP Antibodies

Antigens	Per cent precipitated by	
	Rabbit 1 Anti-λ	Rabbit 2 Anti-λ
γ_2-Globulin*	25.9	29.0
Anti-DNP-BGG 17	0.8	0.7
Anti-DNP-BGG 16	1.2	0.1
Anti-Pipsyl-BGG 4	21.6	26.8
Anti-pipsyl-BGG 1	54.2	60.3
Anti-DNP-OVA 2	0.5	0.9
Anti-DNP-BSA 3	2.7	3.1

* Pentex, Fraction II.

globulin molecules bear L chains type λ. A typical experiment using a commercial preparation of guinea pig γ_2-globulin and involving three successive precipitations of the antigen is shown in Table IV. Six separate aliquots of 0.1 mg of I^{131}-labeled γ_2-globulin were each precipitated by 0.3 ml of the anti-λ-chain antiserum. After 24 hr 0.1 mg of nonlabeled γ_2-globulin and the same volume of antiserum were added to 4 of these tubes. After 48 hr 0.1 mg of cold γ_2-globulin

and 0.3 ml of the antiserum were again added to 2 of these last 4 tubes. It can be seen in Table IV that not more than one-third of the labeled γ-globulin is precipitated by the anti-λ-antiserum even after three successive precipitations. The preparation of γ_2-globulin used in this experiment was at least 95% pure as determined by precipitation with antiserum 5 specific for guinea pig immunoglobulins and reacting with both H and L chains. It should be stressed that if the commercial γ_2-globulin preparation was further purified by DEAE-cellulose under the conditions described in Materials and Methods, only about 25% of the purified fraction could be precipitated by the anti-λ-antiserum. This is due to the fact that in the purification procedure only the more positively charged

TABLE IV

Precipitation of I^{131}-Labeled Guinea Pig γ_2-Globulin by a Rabbit Antiserum Specific for the λ-Type of Guinea Pig L Chain*

Antiserum	No. of precipitations	Precipitated
		%
Rabbit 1, absorbed with anti-DNP antibodies	1	30.4
	2‡	33.1
	3‡	34.1
Rabbit 5, anti-guinea pig antibodies	1	95.0
Normal rabbit serum	1	2.1

* Fraction II (Pentex).

‡ After addition to supernatant of nonlabeled γ_2-globulin and antiserum.

fraction of the γ_2-globulin is recovered. As K and L molecules have different net charges, as illustrated in Fig. 1, the DEAE-purified preparations are enriched in K molecules. Some experiments were also performed with γ_2-globulins isolated from the sera of a few individual guinea pigs of different genetic strains by 50% ammonium sulfate precipitation. In these experiments the amounts of γ_2-globulin and of L molecules were also determined by specific precipitation. The percentages of L molecules obtained with a single precipitation varied from 26 to 33% both in Hartley and strain 13 guinea pigs. A somewhat lower result (22.9%) was obtained in a pool of sera from strain 2 guinea pigs (Table V).

Precipitation Studies with an Anti-κ-Antiserum.—A specific anti-κ-antiserum was more difficult to prepare because of the lack of antibody preparations that contained only L molecules which would be suitable for absorption purposes. An attempt to prepare anti-κ-antisera was made as described in Materials and Methods section (sera 5 and 6). These sera apparently did not contain specific anti-λ-chain antibodies. The immunoelectrophoretic analysis of a mixture of purified κ- and λ-chains showed only one precipitation line when these antisera

were used. Since these sera contained antibodies against H chains of γ_1- and γ_2-antibodies they were exhaustively absorbed with purified H chains obtained from anti-DNP_{65}-BGG antibodies. After absorption they showed a reaction of complete identity between L chains from γ_2-globulin and whole γ_2-globulin by double diffusion in agar. The absorbed antisera precipitated variable amounts of several purified antibody preparations (Table VI) and the percentages precipitated in the same preparation by the anti-κ- and anti-λ-antisera were, as expected inversely related. The anti-κ precipitated as much as 90% of an anti-DNP_{65}-BGG preparation whereas no precipitation was obtained with the anti-

TABLE V

Percentage of Immunoglobulins Bearing the λ-Type of L Chains (L Molecules) in Normal Guinea Pig Immunoglobulins

Guinea pig strain	Individual sera (ammonium sulfate 0 to 50% fraction)	Precipitation given by rabbit anti-guinea pig antibodies	Precipitation given by rabbit anti-guinea pig λ-Chain	Calculated per cent of L molecules
		%	%	
Hartley	1	26.7	7.2	27.0
	2	23.1	6.5	28.2
	3	28.9	9.2	31.8
	4	31.3	8.3	26.5
13	1	23.3	6.2	26.5
	2	21.6	7.1	32.9
2	1*	17.1	3.9	22.9

* Pool of 3 sera.

λ-antiserum. In one of the anti-pipsyl antibodies the anti-κ-serum precipitated only 29.3% of the labeled protein whereas the anti-λ-serum precipitated 81%. The sum of the percentages of protein precipitated by each antiserum in separate aliquots of the same preparation in some instances exceeded 100%. In other experiments it was noticed that if a γ_2-globulin preparation was first precipitated with the anti-κ-serum and then the supernatant reprecipitated with anti-λ-serum, only 15 to 20% of the labeled protein was precipitated by the latter reagent. These results show that the anti-κ-serum may precipitate L molecules.

Electrophoretic Mobility of K and L Molecules.—The precipitation lines given by K and L molecules and by κ- and λ-chains after immunoelectrophoretic analysis (Fig. 1) show that these molecules have a wide range of electrophoretic mobilities. Nevertheless the lines are not parallel throughout. In the slower fractions of γ_2-globulin and L chains there appears to be a lower concentration of L molecules or λ-chains. This was confirmed by the following experiment. A

pool of normal guinea pig serum was subjected to zone electrophoresis in Geon and fractions of proteins having different electrophoretic mobilities were individually labeled with I^{131} and then precipitated with specific antisera. It can be seen in Fig. 6 that there is a fourfold increase in the percentage of L molecules in the anodal fractions over that present in the cathodal fractions. While slow γ_2-globulin contains only about 10% of L molecules, fast γ_2-globulin contains almost 40% of L molecules.

Evaluation of the Molecular Weights of κ- and λ-Chains.—An estimate of the relative molecular weights of the κ- and λ-types of L chains was made by com-

TABLE VI

Precipitation of Guinea Pig Purified Antibody Preparations by Rabbit Antisera Directed Against the κ- or the λ-Types of Guinea Pig L Chains

Purified antibody preparation	Precipitation with anti-κ-serum	Precipitation with anti-λ-serum
	%	%
Anti-pipsyl-BGG3	65.8	29.3
Anti-pipsyl-BGG10	52.5	47.8
Anti-pipsyl-BGG15	62.6	29.2
Anti-pipsyl-BGG14	29.3	81.0
Anti-pipsyl-BGG19	67.0	39.8
Anti-DNP-BGG17	90.5	0.5
γ_2-Globulin*	68.0	24.5

* DEAE-purified fraction II (Pentex).

paring the elution patterns of these chains from a Sephadex column. The following experiment, illustrated in Fig. 7, was performed. Purified guinea pig anti-DNP_{65}-BGG antibodies, containing only K molecules, were labeled with I^{131} and mixed in trace amounts with a preparation of purified guinea pig anti-pipsyl-BGG antibodies which contained equal amounts of K and L molecules. The mixture was extensively reduced in 0.1 M dithioerythritol in the presence of 7 M guanidine·HCl and then filtered through a Sephadex G-200 column equilibrated with 4 M guanidine·HCl in order to separate the H and L chains. The peak of the radioactive κ-chains from the anti-DNP_{65}-BGG antibodies coincides with the optical density peak of the κ- and λ-chains derived from the purified anti-pipsyl antibody preparation. This result shows that both chains have similar molecular weights. It should be noted that the specific activity of the L chains is higher than the specific activity of the H chains when labeling is performed on the whole molecule as in this experiment. It is for this reason that the κ-chain peak from the anti-DNP_{65}-BGG antibodies is much higher than expected considering the H/L relationship (2/1) in the intact molecule.

Since the molecular weights of rabbit γG-immunoglobulin (IgG) H and L chains have been determined by precise methods, another similar experiment was performed in order to compare the elution from the same column of H and

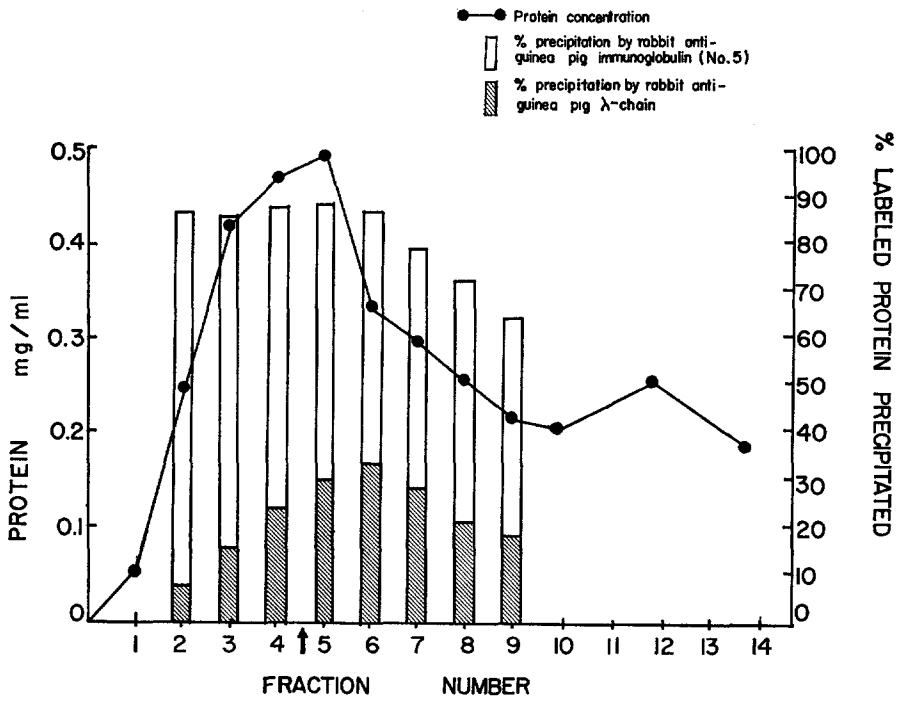

FIG. 6. Percentages of immunoglobulin and of L molecules in different fractions of normal guinea pig serum subjected to zone electrophoresis in Geon block. The protein was eluted from ½-in. segments and the amounts of immunoglobulin and of L molecules were measured in each fraction by specific precipitation. This experiment shows that the slower migrating γ_2-globulin fractions contain a smaller proportion of L molecules than the faster fractions. The anode is to the right of the figure. Note that fractions 7 to 9 eluted from the block contained increasing amounts of serum proteins other than immunoglobulins. The percentages of L molecules in fractions of γ_2-globulin with different electrophoretic mobilities can be calculated as:

$$\frac{\text{per cent labeled protein precipitated by anti-}\lambda}{\text{per cent labeled protein precipitated by anti-immunoglobulin}}$$

These calculated values for fractions 2 to 9 were: 10, 18, 27, 35, 38, 36, 31, and 30%.

L chains from rabbit IgG with H and L chains from guinea pig immunoglobulins. The same preparation of I^{131}-labeled guinea pig anti-DNP_{65}-BGG antibodies was mixed with normal rabbit IgG and the mixture was filtered as above

after extensive reduction. The elution patterns of the H and L chains from both species coincide entirely, which demonstrates that they have similar molecular weights (Fig. 8).

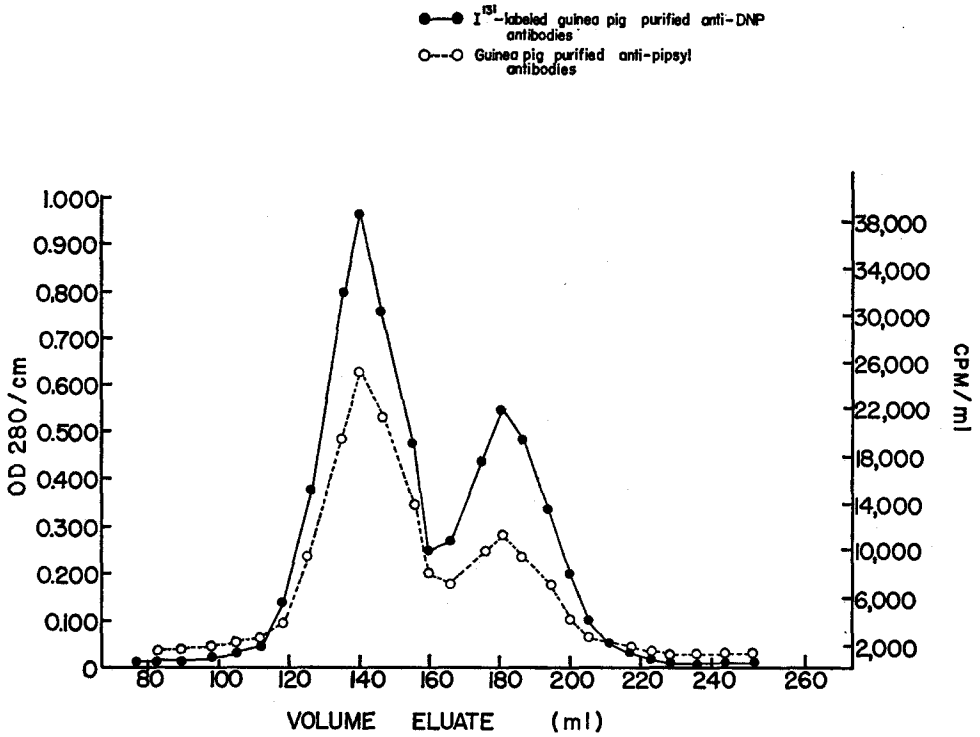

FIG. 7. Filtration through Sephadex G-200 in 4 M guanidine · HCl of extensively reduced and alkylated guinea pig antibodies (26 mg of cold anti-pipsyl-BGG and 0.2 mg of I^{131}-labeled anti-DNP_{65}-BGG. Open circles: optical density of the H and L (half κ, half λ) from the anti-pipsyl antibodies. Closed circles: radioactivity of the H and L (κ) chains from the anti-DNP antibodies. This experiment shows that κ- and λ-chains have similar molecular weights.

DISCUSSION

Two distinct types of L chains have been described in human (14, 15) and mouse immunoglobulins (16, 17), and in this paper we present evidence that the same occurs in guinea pig immunoglobulins as had been previously suggested (18).

In the case of human immunoglobulins it was found that both L chain types (κ and λ) are found in all classes of immunoglobulins and that the relationship of K/L molecules in normal human beings is 2/1. Moreover these different types of L chains are known to be synthesized in different cells (19) and to differ

widely in the peptides (20) which form the C terminal portion of the chain (21, 22).

The properties of the two different types of mouse L chains differ in at least one important respect from those of their human counterparts. Studies on mouse myeloma proteins have shown that only one type of L chain was as-

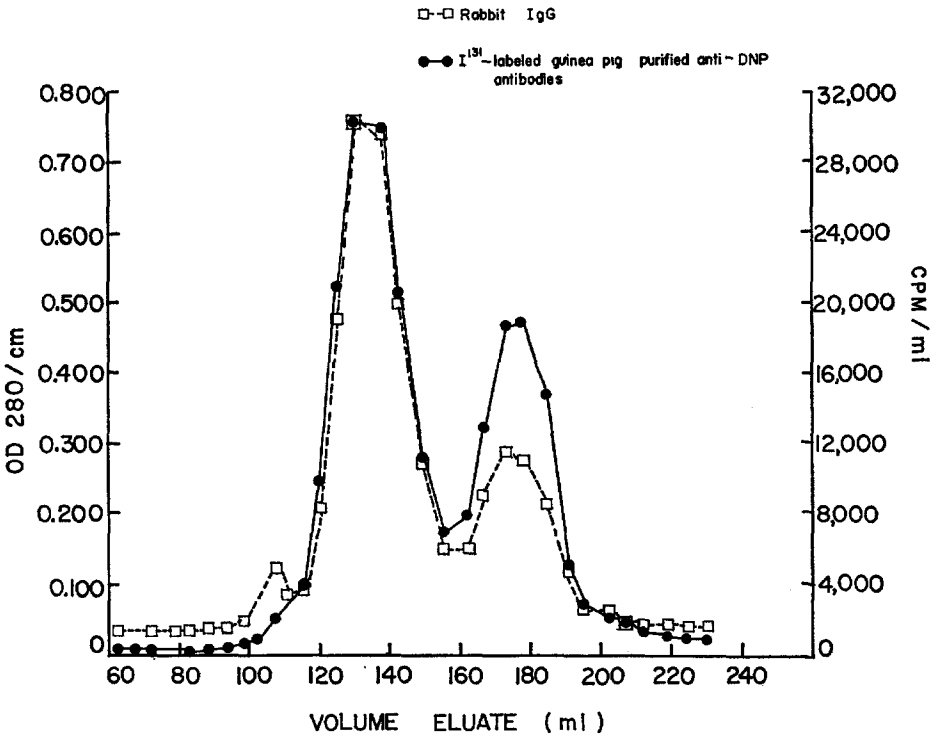

FIG. 8. Filtration of extensively reduced and alkylated cold rabbit IgG (20 mg) and I^{131} guinea pig anti-DNP-BGG antibodies (0.3 mg) through Sephadex G-200 in 4 M guanidine · HCl. This experiment shows that rabbit and guinea pig H and L chains respectively are similar in size.

sociated with all γG-Be1, γG-Be2, γA, and γF (γ_1) myeloma proteins. A different type of L chain has recently been isolated from a γM-myeloma protein (17).

In the guinea pig, as demonstrated in the present study, the situation seems to be analogous to that found in human immunoglobulins. Both types of L chains (κ and λ) are found in γ_1- and γ_2-classes of guinea pig immunoglobulins and L molecules represent about one-third of the population of normal γ_2-globulin, while the majority or all of the others are K molecules.

The quantitative studies on guinea pig L molecules were done by specific precipitation of I^{131}-labeled antigens and were greatly facilitated by the ob-

servation that antibodies prepared against DNP_{65}-BGG contain only K molecules (13), which allowed the preparation of an anti-λ-antiserum. The direct measurement of the amounts of K molecules, performed by the same method, was subject, however, to some doubt because of uncertainties regarding the specificity of the antisera used. These antisera (Nos. 5 and 6) were prepared in rabbits by the injection of small amounts of κ-chain and of purified anti-DNP_{65}-BGG antibodies (K molecules) and were thoroughly absorbed with H chains obtained from the same antibody preparation. Only anti-κ-chain antibodies were detected in this serum by means of double diffusion in agar and immunoelectrophoresis. The possibility cannot be excluded, however, that this antiserum contained small amounts of specific anti—λ-antibodies. The presence of such contaminating anti-λ-antibodies would account for the observation that in a few instances more than 100% was found by adding the percentage of immunoglobulin precipitated by anti-κ- and anti-λ-antisera in separate aliquots of the same preparation. Anogher explanation for this observation would be that κ- and λ-chains share common antigenic determinants. If κ- and λ-chains are indeed shown to share common amino acid sequences in their C terminal portions, this might mean that the corresponding genes have arisen one from the other by duplication and independent mutation.

Guinea pig κ- and λ-types of L chain are electrophoretically heterogeneous. Certain differences in mobility could, however, be detected in the slower fraction of γ_2-globulin which were found to contain a greater concentration of K molecules than the total population of γ_2-globulin.

Although no clear demonstration of the presence of different types of L chains has yet been made in rabbit immunoglobulins, two types of IgG are known to exist in this species. These two types of IgG are differentiated by their net charge and therefore distinct electrophoretic mobilities. At least part of this difference in charge seems to be related to the L chains they contain (23). These different rabbit L chains might be homologous to the guinea pig and human L chains. More thorough studies of possible antigenic differences in the L chains from fractions of rabbit immunoglobulins with different electrophoretic mobility might clarify this problem.

The variability of the L polypeptide chains (16, 21, 22) which is a unique and important characteristic of immunoglobulins, is probably related to the specificity of antibodies (2, 24–28). This variability occurs in the N terminal part of the chain. It is possible, however, that evolutionary changes in the C terminal portion of the chain have increased the range of configurations that immunoglobulins can assume for their function, and this could constitute an important selective advantage.

SUMMARY

Guinea pig 7S immunoglobulins (γ_1 and γ_2) consist of two groups of molecules (K and L) bearing different types of L chains (κ and λ). Approximately

one-third of the molecules in normal guinea pig γ_2-globulin bear the λ- type of L chains, and all or most of the others bear κ-chains. κ- and λ-chains have similar molecular weights.

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