Comparison of Polypeptide Chains of γG-Globulin from Bursectomized and Normal Chickens.* (32139)

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The bursa of Fabricius is the major organ of immunological competence in the chicken (1). Homograft immunity remains essentially intact in bursectomized chickens(2-4), whereas these birds produce either no or reduced amounts of antibody(1,5,6). It has been suggested that the role of the bursa in immunological maturity may involve release of specialized cells(7), or of specific chemicals, perhaps hormonal in nature(8).

The structure of chicken γG-globulin(9, 10) is consistent with the 4-chain structure proposed by Porter(11), involving one pair of light (L) chains and one pair of heavy (H) chains held together by inter-chain disulfide bonds. Although bursectomized birds synthesize reduced amounts of antibody, they may produce as much as 40% of the normal amount of γG-globulin, and this may represent "non-antibody" γG-globulin(12). It is possible that the decreased antibody synthesis results from the synthesis of altered or incomplete globulin subunits. Thus, the purpose of this investigation was to determine whether γG-globulin produced by bursectomized chickens is structurally different from globulin produced by normal chickens of the same age. Evidence is presented that the subunit structure of γG-globulin from normal and from bursectomized chickens is the same, and that chicken H chains can be resolved into 10-12 bands by electrophoresis on urea-starch gel by the procedure of Sjöquist(13).

Methods and materials. Bursectomy. Chickens were chemically bursectomized either by dipping 3-day-old embryonated eggs (Kimber Farms Strain K44) in 1280 mg of testosterone propionate (Nutritional Biochemical Corp.), contained in 100 ml ethyl alcohol(14), or by injecting 4 mg of testosterone propionate in 0.1 ml corn oil (Mazola) into the chorioallantoic cavities of 13-day embryos(15). Almost normal hatches were obtained with either procedure. Control chickens were treated as above, except that testosterone propionate was absent.

Purification of γG-globulin. Normal and bursectomized 6-week-old chickens were bled and individual sera were analyzed by agar immunoelectrophoresis(16). Only sera which showed reduced amounts of γG-globulin on immunoelectrophoretic analysis, and which were obtained from chickens that had no visible bursa upon autopsy, were used. Sera from individual birds were precipitated 3 times with sodium sulfate(17) and dialyzed against pH 8.2 borate buffer. The γG fractions were obtained by gel filtration in Sephadex G-200 columns (4.5 × 40 cm) and elution with borate buffer (pH 8.2, $\Gamma/2 = 0.16$). A decrease of approximately 40% by weight of the γG-globulin fraction was obtained from sera of bursectomized chickens.

Purification of H and L chains. The γG-globulin preparations were reduced with mercaptoethanol and alkylated with iodoacetamide according to the procedures of Fleischman *et al*(18). For preparation of L chains, the alkylated γG-globulin was dialyzed against 1 M propionic acid and the L Chain fraction was recovered by elution from Sephadex G-200 columns with 1 M propionic acid. After elution, the L chain fraction was dialyzed against water and lyophilized.

Heavy chains were prepared by elution with 0.1 M formic acid, employing the procedure of Sjöquist(13). After alkylation, the γG-globulin preparations were dialyzed against 0.1 M formic acid and eluted from a Sephadex G-200 column with 0.1 M formic acid. The separated polypeptide chains were dialyzed against water and lyophilized.

Starch gel electrophoresis. Alkaline starch gel electrophoresis was performed with 6 M urea in pH 8.8 borate buffer in the presence

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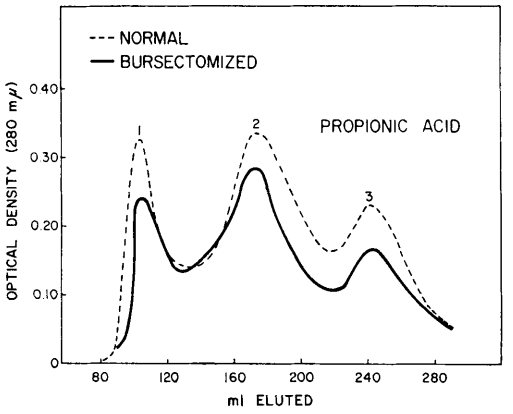

FIG. 1. Elution from Sephadex G-200 in 1 M propionic acid of reduced and alkylated γG-globulin from normal and bursectomized chickens.

of 0.035 M glycine(19). Lyophilized protein (1.5 mg) was dissolved in 0.1 ml of 0.3 M pH 8.8 borate buffer made 10 M in urea. The samples were applied to the gel, which contained 67.2 g of starch, 176.4 g of urea, and 366 ml of glycine buffer pH 7.6. The electrode vessels contained 0.3 M borate buffer, pH 8.8. Electrophoresis was performed for 12-22 hours at 6-8 V/cm. After electrophoresis the proteins were stained with Amido Black. Acid starch gel electrophoresis was performed in 6 M urea, pH 2.9(10). The procedure for preparing the gel was similar to that outlined above; however, the gel buffer was 0.05 M sodium formate-formic acid, pH 2.9. The electrode vessels contained 0.2 M solution of the formate buffer.

Results. Gel filtration on Sephadex G-200 in propionic acid of reduced, alkylated γG-globulin from normal and bursectomized birds resulted in identical elution patterns (Fig. 1). In order of elution, the 3 fractions were identified by Dreesman and Benedict(9) and Gold *et al*(10) as aggregated H chains, H chains, and L chains. Lyophilized fractions were analyzed by urea-formate starch gel electrophoresis (Fig. 2B). The L chain fraction (Fig. 1, peak 3) migrated most rapidly to the cathode, and in this respect resembled L chains of other species(20). As with H chains of other species, the H chain fraction (peak 2) migrated less rapidly. Peak 1 had a slower migration. In the patterns obtained in the acid starch gel, there were no discernible differences between the chains of γG from normal and from bursectomized chickens.

The L chain bands of the bursectomized and the normal chickens were compared in alkaline gels. As is shown in Fig. 3A, there were 10-12 bands. The patterns of the individual bands in the bursal and normal samples appeared identical in position and intensity. In the alkaline gel, the H chains migrated as a diffuse band.

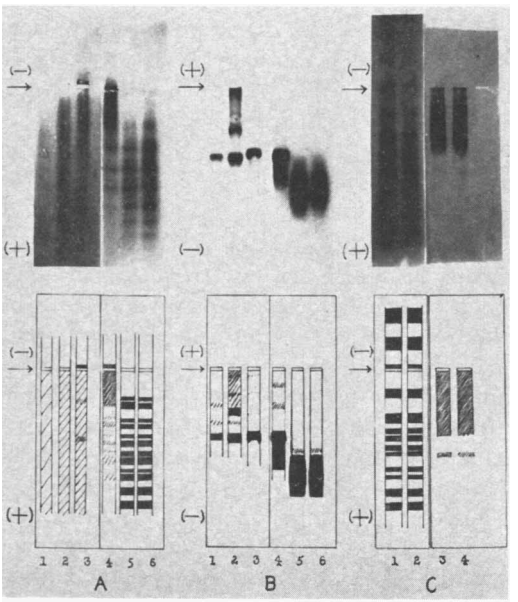

FIG. 2. A. Electrophoretic patterns in urea-starch gel at pH 8.8 of reduced and alkylated γG-globulin chains from 6-week-old normal and bursectomized chickens. The chains were prepared by gel filtration in propionic acid. Nos. 1, 3, 5, contain aggregates, H chains, and L chains, respectively, of γG-globulin from normal chickens (see Fig. 1). Nos. 2, 4, 6, contain aggregates, H chains, and L chains, respectively, of γG-globulin from bursectomized chickens. (see Fig. 1).

FIG. 2. B. Electophoretic patterns in urea-formic acid-starch gel at pH 2.9 of reduced and alkylated γG-globulin chains from 6-week-old normal and bursectomized chickens. Nos. 1, 3, 5, contain aggregates, H chains, and L chains, respectively, of γG-globulin from normal chickens (see Fig. 1). Nos. 2, 4, 6, contain aggregates, H chains, and L chains, respectively, of γG-globulin from bursectomized chickens (see Fig. 1).

FIG. 2. C. Electrophoretic patterns in urea-starch gel at pH 8.8 of reduced and alkylated γG-globulin chains from 6-week-old normal and bursectomized chickens. The chains were prepared by gel filtration in formic acid. Nos. 1 and 3 contain H and L chains, respectively, from normal chickens; Nos. 2 and 4 contain H and L chains, respectively, from bursectomized chickens.

Separation of the H and L chains on Sephadex G-200 with 0.1 M formic acid resulted in 3 peaks, similar to those obtained by elution with propionic acid. The γG-globulin from normal and from bursectomized chickens had elution patterns similar to those obtained in propionic acid columns. The lyophilized fractions from these columns were applied to alkaline starch gel for electrophoresis. The L chains migrated most rapidly to the anode. The H chains (peak 2) were resolved into 10-12 bands (Fig. 2C), and in this respect resembled the H chains of other animal species(20-22). No difference in either the intensity or the position of the H-chain bands was observed between bursectomized and normal chickens. Electrophoresis of the H chains on urea-formate starch gel did not resolve the H chains. It is interesting to note that, in contrast to human gamma globulin (13), poor phenograms of H chains were obtained when iodoacetic acid was used for alkylation of chicken gamma globulin. Also, in contrast to human gamma globulin(23), no banding of chicken γG-globulin H chains was obtained with S-sulfonation.

Discussion. Bursectomized chickens have been shown to form small amounts of specific antibody(5-7) and to synthesize reduced amounts of gamma globulin. We have observed a reduction of approximately 40-60% of γG-globulin in chickens which lacked visible bursae Whether most of the γG-globulin present in bursectomized birds represents non-antibody γG-globulin or the sum of small amounts of antibody formed in response to a variety of antigens, is not known. In discussing the role of the bursa in antibody formation, Carey and Warner(12) have pointed out that other experimental conditions could result in the formation of non-specific globulin of unknown nature, namely, a rise in excess of detectable antibody synthesis when antibody synthesis is stimulated(24), and an increased production of γG-globulin in rabbits which had been irradiated 24 hours prior to antigenic stimulation(25). These examples of globulin of unknown specificity might be analogous to the globulin present in bursectomized chickens.

There seems to be little doubt of some type of subunit interaction in the formation of active antibody. Reconstitution experiments demonstrate that most L and H chains will pair with each other *in vitro*(26-28) by means of interacting regions of the chain. The interaction of L with H chain suggests that antibody molecules may be formed by self-assembly from chains *in vivo,* and that antibodies of different specificities may be formed from different subsets of L and H chains having different amino acid sequences. If we assume the presence in the normal chicken of these subsets, then the deficiency in antibody in bursa-less chickens apparently is not inherent in the synthesis of L or H subunits, since the phenograms of γG-globulin from normal and bursectomized birds were identical. It has been suggested (8,29) that a soluble factor of normal bursae is necessary for maturation of competent cells in bursa-less chickens. If these cells cannot express their competence by production of antibody in the absence of bursal factors, this defect is not due to their inability to produce H or L chain subunits.

Until the bursal substance is chemically identified and the site of reaction is elucidated, the mechanism by which the bursa might exert control of antibody synthesis is only speculative. The simplest explanation for the synthesis of small amounts of detectable antibody in bursectomized birds is that there is simply a reduction in the number of those cells involved in antibody formation(4,12); however, it is also possible that a defect exists in the mechanism of gamma-globulin synthesis(6). It seems reasonable to assume that this defect, if present in bursectomized chickens, would not occur at the translation or transcription step in protein synthesis, since we observed the same heterogeneous populations of H and L chains in both normal and bursa-less chickens. This would imply that either H or L subunits are not put together correctly after formation at the polysome level(30), or that the defect might exist in the processing of antigen prior to the reading or activation of the genome.

Summary. Reduced and alkylated γG-globulin from bursectomized and normal chickens had the same elution pattern from Sephadex

G-200 columns when either propionic acid or formic acid was used for elution. The phenograms obtained from column-separated polypeptide chains were compared, and no difference was found in either the L chain or H chain banding patterns of γG-globulin from bursectomized and normal chickens. These results indicate that the subunit of γG-globulin is the same for bursectomized and normal chickens.

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Production of Anti-*Mycoplasma* (PPLO) Antibodies in Rabbits.* (32140)

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Several methods have been used to immunize rabbits with *Mycoplasma* (pleuropneumonia-like organisms, PPLO). These reported procedures ignore quantitation of the protein content of the antigens injected. Frequently the resulting immune sera had relatively low agglutinin titers.

This communication reports experiences with various routes of antigen introduction into rabbits and the results with foot pad inoculation with quantitatively controlled amounts of antigen.

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