DEVELOPMENT OF CHICKEN LYMPHOID SYSTEM

I. Synthesis and Secretion of Immunoglobulins by Chicken Lymphoid Cells*

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In the chicken, the bursa of Fabricius is essential for the development of lymphoid cells that will synthesize and secrete both IgM and IgG (1–3). When newly hatched chickens are bursectomized and irradiated, they fail to synthesize serum immunoglobulin(s) (Ig)[1] even though the expression of cell-mediated immunity remains normal. Hence, the bursa of the chicken provides an excellent experimental model to study the development of the lymphoid cells which synthesize circulating Ig.

The present report describes the synthesis and secretion of Ig by lymphoid cells obtained from the spleen, bursa, and thymus of the chicken. Both spleen and bursa cells synthesize IgM and IgG as defined by serology and acrylamide gel electrophoresis. Kinetics of synthesis and secretion of Ig by normal spleen cells was not greatly different from that of mouse myeloma cells as previously reported (4). In addition to synthesizing and secreting IgG, the bursa cells synthesize IgM which apparently is not secreted. The structure of this IgM of the bursa cells was studied by acrylamide gel electrophoresis and found to be different from Ig synthesized by the lymphoid cells of the spleen which are differentiated from the bursa. Ig was not detected in thymus cells within the limits of the method used.

Materials and Methods

Animals.—White Leghorn chickens, line 91, were obtained as fertilized eggs from the Basic Laboratory, Hy-Line Poultry Farms (Johnston, Iowa), through the courtesy of Dr. G. R. Law. Eggs were incubated in a Jamesway Model 252 incubator (James Mfg. Div., Fort Atkinson,

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[1] *Abbreviations used in this paper*: B cells, bursa-dependent or bone marrow–dependent cells; BGG, bovine gamma globulin; Ig, immunoglobulin; KLH, keyhole limpet hemocyanin; NP-40, Nonidet P-40; SDS, sodium dodecyl sulfate; T cells, thymus-dependent cells; TKM, 0.05 M Tris–HCl (pH 7.6, 4°C)–0.025 M KCl–0.005 M $MgCl_2$.

Wis.). The age of the chickens used for the experiments was 6-12 wk. For immunization, chickens were injected subcutaneously with 5 mg of bovine gamma globulin (BGG, Sigma Chemical Co., St. Louis, Mo.) in 0.5 ml of complete Freund's adjuvant every 2 wk. A total of three to four doses was given to each animal. Lymphoid cells were obtained from the chicken 3–5 days after the last dose.

Incorporation of Radioisotopes.—Experiments on the kinetics of Ig synthesis and secretion were performed with cell suspensions prepared from the lymphoid tissues as previously described in the experiments with mouse myeloma cells (4). It is essential for defining precursor-product relations that the cells maintain a constant rate of protein synthesis during the length of time needed to establish a state of equilibrium. When the bursa cells were incubated at a concentration of 2×10^7 cells/ml in a leucine-less Eagle's medium (5), containing 5.0% fetal calf serum and 20 μCi/ml L-leucine-(4,5-^{3}H) (40 Ci/mmole; Schwarz Bio Research Inc., Orangeburg, N. Y.), the rate of protein synthesis was constant for at least 6 hr. Incubations were performed in a humid tissue culture incubator at 37°C in 15% CO_2–85% air.

For continuous-labeling experiments, the cell suspensions were prewarmed to 37°C before adding radioactive amino acids. Aliquots were then distributed into individual Petri dishes and placed in the incubator. At each sample time the incubation mixture was transferred to a centrifuge tube, quickly chilled in an ice-water bath, and centrifuged at 3000 *g* for 10 min to separate the cells from the supernatant. The cell pellet was suspended in 0.05 M tris(hydroxymethyl)aminomethane (Tris)–HCl (pH 7.6, 4°C)–0.025 M KCl–0.005 M $MgCl_2$ (TKM), lysed by adding Nonidet P-40 (NP-40; Shell Chemical Co., New York) to a final concentration of 0.5%, and the nuclei and ribosomes were removed by centrifuging at 105,000 *g* for 120 min at 4°C (6). Both the cell lysate and supernatant fractions were then divided into three aliquots. Two aliquots were assayed serologically and the remaining one was used for determining trichloroacetic acid (TCA)—precipitable radioactivity.

The lymphoid cells from the spleen were prepared differently. The cell suspensions prepared by mechanical disintegration of the tissue and filtration were brought to a concentration of $\backsim 10^8$ cells/ml and allowed to stand at 37°C for 45–60 min to sediment erythrocytes. The cells which did not sediment with erythrocytes were used for the incorporation experiment. The concentration of the spleen cells used for the kinetic experiment was 2×10^7 cells/ml. Preliminary experiments showed that this concentration of the cell suspension exhibited the most efficient incorporation of radioactive amino acids with a linear rate of protein synthesis.

Preparation of Antisera and Serological Assay.—Antisera against chicken Ig were prepared in rabbits by injecting purified chicken IgG and IgM. For immunization, chicken IgM and IgG were prepared as follows: adult chickens were stimulated by injections of (about 10^{10} organisms) DNP-*Brucella* per chicken, in complete Freund's adjuvant (7). Immune chicken serum was absorbed by DNP-Sepharose and eluted by 0.2 M glycine-HCl buffer (pH 2.8) (8, 9). IgM was separated from IgG by gel filtration through a Sephadex G-200 column (2.5 × 100 cm, Pharmacia Fine Chemicals Inc., Uppsala, Sweden) in 0.015 M Tris-HCl buffer (pH 7.4) with 0.14 M NaCl (19). Chicken Ig thus purified exhibited a single band in immunoelectrophoresis against a rabbit antiserum prepared against whole chicken serum. The purity of IgM and IgG was further verified by demonstrating a single band in rabbit antiserum prepared against these purified antigens.

Anti-μ antiserum was prepared from anti-IgM by a solid immune adsorption technique with polymerized chicken IgG by the method of Avrameas and Ternynck (10). Anti-γ antiserum was also prepared by the above method.

Anti-KLH (keyhole limpet hemocyanin) antiserum was prepared by injecting rabbits with KLH prepared as previously described by Campbell et al. (11). This antiserum did not react with any chicken serum component in double-diffusion agar.

Antiserum to rabbit IgG was prepared in goats. The rabbit IgG used as antigen was prepared by the method of Fleischman et al. (12).

Chicken Ig labeled with radioactive amino acids were complexed with an excess of rabbit anti-chicken IgM serum and the complexes were precipitated by goat anti-rabbit IgG. This indirect precipitation technique was used throughout. Titrations of the rabbit anti-chicken Ig and of the goat anti-rabbit IgG were performed using leucine-^{3}H–labeled Ig that had been secreted by the chicken spleen cells. The detailed method of preparation of serological precipitates for quantifying radioactive antigen was described elsewhere (13).

Acrylamide Gel Analysis.—The method of sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis of serological precipitates was described in detail elsewhere (13). The immune precipitates were collected by centrifugation, washed three times with phosphate-buffered saline at 4°C, and dissolved in 0.3 ml of 10 M urea–1% SDS–0.5 M Tris (pH 8.5). For reduction of precipitates, 2-mercaptoethanol was added to a final concetration of 0.2 M and the mixture was incubated for 3 hr at 37°C. Iodoacetamide, 0.5 M in 2 M Tris, pH 8.5, was added for alkylation to a final concentration of 0.25 M and incubation was continued for 60 min at 37°C. The samples were dialyzed overnight at room temperature against 0.01 M phosphate buffer, pH 7.2, containing 0.1% SDS and 0.5 M urea. Reduced and alkylated samples were dialyzed against the same buffer with 0.2 M 2-mercaptoethanol. 0.05–0.1 ml dialyzed samples were electrophoresed in acrylamide gels at 8 ma/gel for 3.5 hr, fractionated, and the radioactivity was counted as described previously (14, 15).

RESULTS

Serological Precipitation of Leucine-^{3}H–Labeled Cytoplasmic Extract and Acrylamide Gel Electrophoresis.—The serological assay using indirect precipitation was shown to be very useful in quantifying Ig synthesized by the mouse myeloma cells (13, 16, 17). In contrast to the myeloma cells which synthesize and secrete relatively large quantities of monoclonal Ig, nonspecific radioactivity precipitated by anti-KLH was significantly high when the serology was performed with radioactive proteins of the normal lymphoid cells. Hence, the specificity of serological assay was carefully studied by quantifying radioactivity precipitated by specific and nonspecific antisera and by analyzing serologically precipitated proteins in acrylamide gel electrophoresis as described below.

An aliquot of the cell suspension prepared from the spleen was labeled with leucine-^{3}H for 3 hr and then centrifuged at 3000 *g* for 10 min to separate the cells from the incubation media. The cell pellet was suspended in TKM buffer, solubilized in 0.5% NP-40 TKM, and the nuclei were separated from the cytoplasm by centrifuging at 3000 *g* for 10 min (6). The cytoplasm was further centrifuged at 105,000 *g* for 2 hr to sediment the ribosomes. The resultant cell extract and the incubation media containing the secreted proteins were both subjected to serological precipitation and acrylamide gel electrophoresis. As shown in Fig. 1 *a*, no distinct peak was observed in the gel of nonspecific precipitates, whereas the specific precipitates showed a distinct peak of chicken Ig as well as background radioactivity. To eliminate background radioactivity, nonspecific precipitation with anti-KLH serum was first performed, and then the supernatant of this nonspecific serology was subjected to specific precipitation with anti-chicken IgM. Acrylamide gel electrophoresis of this specific precipitate

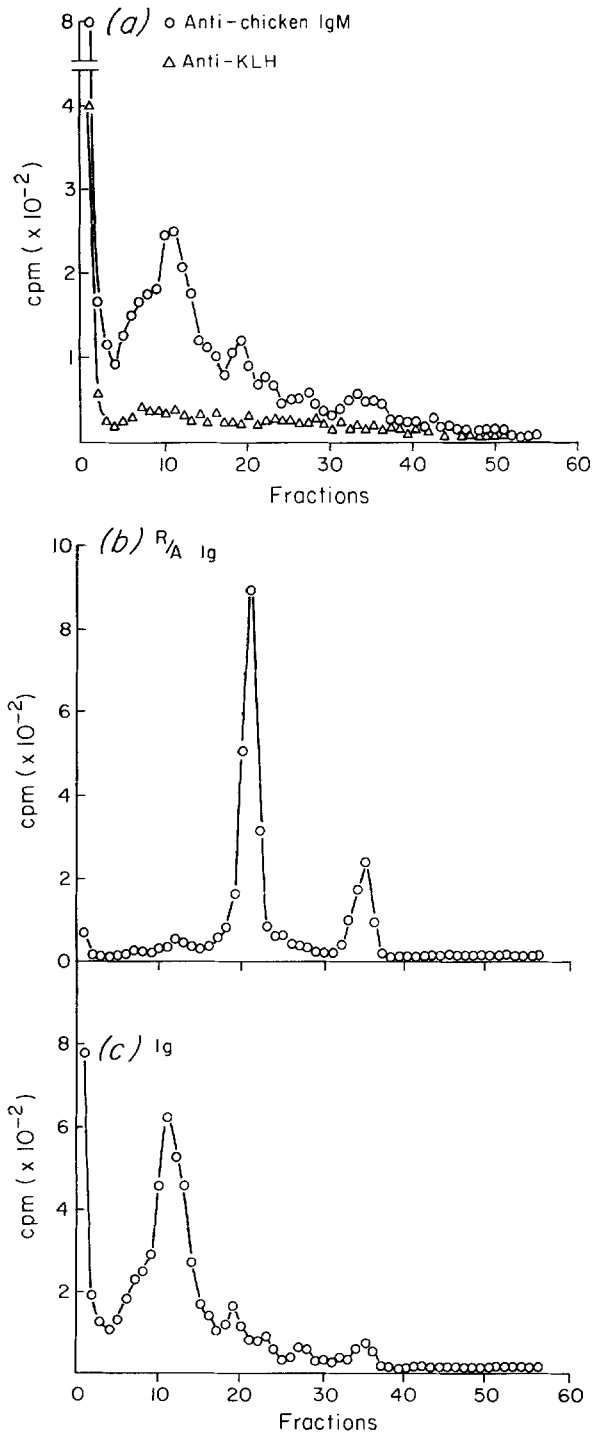

FIG. 1. Specificity of serological precipitation assay analyzed by acrylamide gel electrophoresis. Intracellular proteins were labeled with leucine-^{3}H by incubating the spleen cells for 3 hr as described in Materials and Methods. The detergent-soluble fraction of cells was subjected to serological precipitation and analyzed by SDS–acrylamide gel electrophoresis. Fractions were numbered from the negative to the positive electrode. Specific precipitates with

exhibited more remarkable peaks of IgM and IgG (Fig. 1 *c*). This "clean-up" procedure did not result in loss of specific radioactivity attributable to loss of Ig. These Ig peaks were further verified by acrylamide gel electrophoresis after reduction and alkylation of the same serological precipitates, which showed that more than half of the radioactivity found in IgM and IgG was recovered in the heavy (H) and light (L) chain peaks (Fig. 1 *b*).

The molecular weights of IgM, IgG, H, and L chains were also determined on SDS-acrylamide gels (Fig. 2) (18). The molecular weights thus determined were as follows: IgG, ~170,000; H chain, ~70,000; L chain, ~23,000. IgM did not migrate into the gel. These results agree with the values previously reported for chicken Ig purified from serum (19).

Based upon the above experimental results, leucine-^{3}H–labeled Ig were

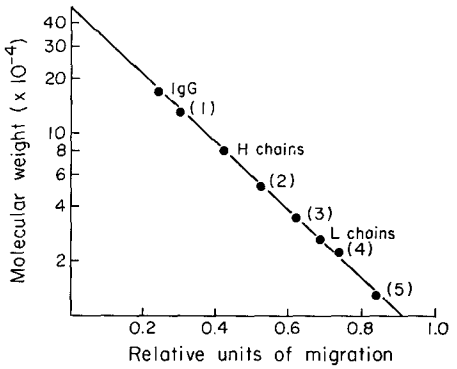

FIG. 2. Molecular weight determination from SDS–acrylamide gel electrophoresis. Migration of chicken IgG and H and L chains is an average of 5–10 experiments. Standards are (1) *E. coli* β-galactosidase; (2) chicken albumin; (3) porcine pepsine; (4) trypsin; (5) cytochrome *c*.

quantified by a two-step serological assay as follows. An aliquot of leucine-^{3}H–labeled cytoplasm which was solubilized by NP-40 was first subjected to nonspecific precipitation. The supernatant from the above was divided into two aliquots for specific and nonspecific serology. The difference in radioactivities between them was assumed to represent the Ig. Nonspecific radioactivities were found to be very small in the secreted proteins which contained more than 75% leucine-^{3}H–labeled proteins as Ig.

Synthesis and Secretion of Ig by the Spleen Cells.—The kinetics of synthesis and secretion of Ig were studied by incubating a cell suspension with leucine-

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anti-IgM, ○; nonspecific control precipitates with anti-KLH, △; (*a*) comparison of specific and nonspecific precipitates; (*b*) reduction (*R*) and alkylation (*A*) of the specific precipitate from (*c*); (*c*) specific precipitate on the supernatant fraction obtained after nonspecific precipitation with anti-KLH.

^{3}H to label the newly synthesized proteins. As shown in Fig. 3 *a*, the incorporation of leucine-^{3}H into trichloroacetic acid–precipitable material proceeds at a constant rate for 5 hr. The conditions of incorporation were chosen after preliminary experiments varying cell concentration and fetal calf serum content

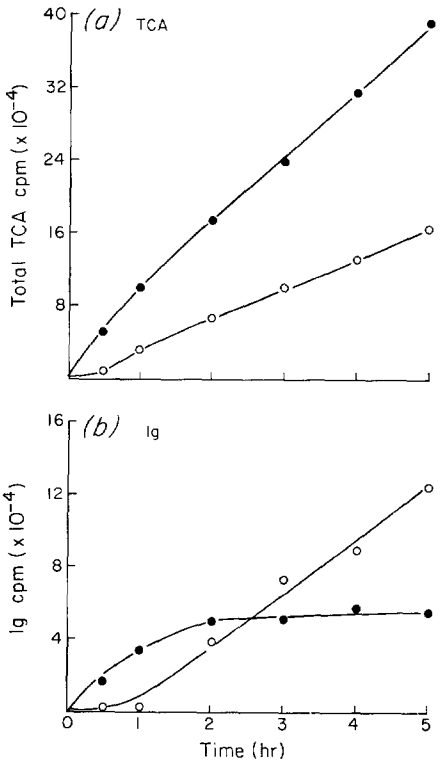

FIG. 3. Kinetics of incorporation of leucine-^{3}H into trichloroacetic acid–precipitable material and Ig of the spleen cells. A cell suspension (2×10^7 cells/ml) in leucine-less Eagle's medium containing 5% fetal calf serum was incubated at 37°C with leucine-^{3}H (16 μCi/ml). Aliquots of 1 ml each were distributed into petri dishes and the experiment was performed as described in Materials and Methods. Intracellular protein, ●; secreted protein, ○; (*a*) trichloroacetic acid–precipitable material; (*b*) serologically precipitable Ig.

in the incubation mixture in order to ensure a linear synthesis of cellular proteins.

The kinetics of incorporation of leucine-^{3}H into Ig were different from incorporation into total trichloroacetic acid–precipitable material (Fig. 3 *b*). The amount of labeled Ig inside the cell increased without lag for about 3 hr and then remained constant, indicating saturation of the intracellular pool. Labeled Ig were detectable in the medium outside the cell after a lag of 30 min. The rate of secretion increased and became constant after 2 hr. After 3 hr, the

amount of secreted Ig became larger than the intracellular pool. In the medium outside the cells, Ig accounted for 75–80% of total trichloroacetic acid–precipitable radioactivity.

Table I shows the relative amounts of Ig and total proteins synthesized and secreted by the spleen and bursa cells at 4 hr of incubation. In the spleen of the chicken stimulated with BGG, 74% of the secreted proteins and 25% of the intracellular proteins were serologically precipitable Ig, which is comparable to that of mouse myeloma cells (13). When the chicken was not stimulated by BGG, the amount of Ig in both secreted and intracellular proteins was lower (i.e., 50 and 5%). Such antigenic stimulation, however, had no demonstrable effect on the capacity of the bursa cells to synthesize and secrete Ig (Table I). Synthesis of Ig by the bursa cells will be further discussed below.

TABLE I

Leucine-^3H Incorporation into Immunoglobulins and Total Proteins

Tissue		cpm × 10^{-3}*		Ig/TCA
		TCA	Ig	
				%
Spleen	Secreted	83.3	31.8	38
	Intracellular	786.8	32.6	4
Spleen (BGG)‡	Secreted	99.2	73.7	74
	Intracellular	191.3	47.6	25
Bursa	Secreted	26.7	5.4	20
	Intracellular	394.2	6.6	2
Bursa (BGG)	Secreted	52.8	4.9	9
	Intracellular	448.8	5.7	1

* Per 1.5 × 10^7 cells.

‡ Spleen cells from the chicken which has been sensitized by bovine gamma globulins.

The high specific activity of Ig in the secreted proteins was also verified by analyzing secreted material in SDS-acrylamide gel (Fig. 4 *b*). A protein peak of the molecular size of IgG (fraction No. 12–14) is the major component in the secreted material. This peak was shown to be IgG by acrylamide gel electrophoresis of serological precipitates with anti-Ig (Fig. 4 *a*).

Synthesis and Secretion of Ig by the Bursa Cells.—Fig. 5 shows the kinetics of synthesis and secretion of Ig by the bursa cells which is different from that of the spleen cells (Fig. 3 *b*). The intracellular Ig pool of the bursa cells was not saturated with leucine-^3H–labeled Ig and continued to increase throughout the incubation period. The amount of secreted Ig did not become greater than that synthesized inside the cells, suggesting that part of the Ig synthesized by the bursa cells was not secreted. Small amounts of specific radioactivity which were not secreted are Ig (Table I). The specific radioactivity could always be reduced to the level of that precipitated with nonspecific antisera by adding excess

purified IgM to the serological reaction, verifying the specificity of the serological assay in the lymphoid cells where very small amounts of Ig are synthesized.

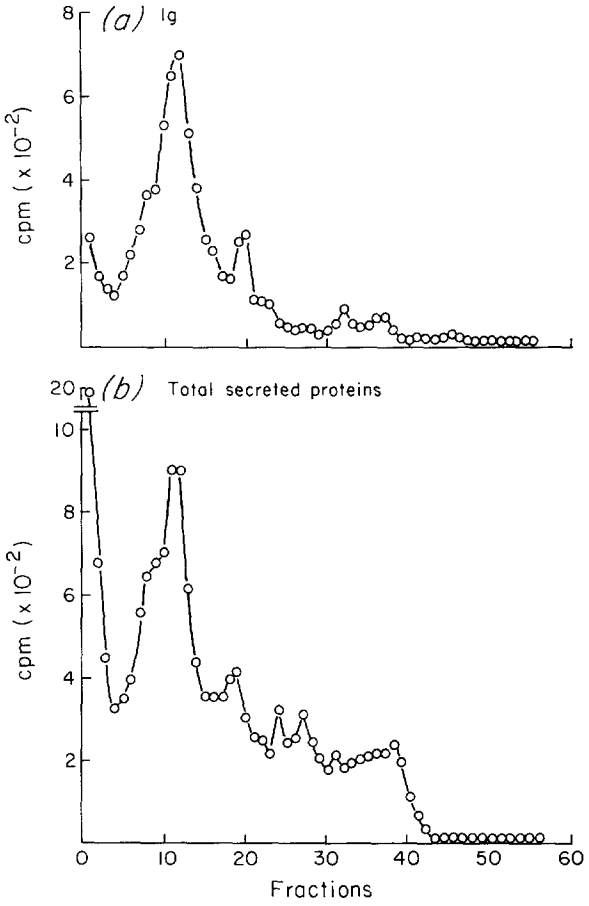

FIG. 4. Acrylamide gel electrophoresis of leucine-^{3}H-labeled proteins secreted by the spleen cells. (*a*) Serological precipitates with anti-IgM of the incubation media; (*b*) trichloroacetic acid–precipitable material of incubation media.

As shown in the following section, the Ig which is not secreted from the bursa cells have a different structure from that which is secreted.

Structural Characteristics of Ig Synthesized by the Bursa Cells.—Ig synthesized by the bursa cells were studied by SDS–acrylamide gel electrophoresis and compared with Ig synthesized by the spleen cells using ^{3}H/^{14}C double labeling. The lymphoid cells from the bursa were incubated with leucine-^{3}H for 3–4 hr to label all intracellular Ig, and the spleen cells were labeled with L-leucine–

$^{14}C(U)$ (262 mCi/mM; New England Nuclear Corp., Boston, Mass.). The radioactive proteins synthesized and secreted by two populations of the lymphoid cells were mixed in an adequate ratio of $^{3}H/^{14}C$, subjected to serological precipitation with anti-IgM, and analyzed in SDS–acrylamide gel electrophoresis.

Fig. 6 compares the acrylamide gel electrophoresis of the Ig secreted by the spleen and bursa cells. Compared to the spleen cells which secrete Ig with a

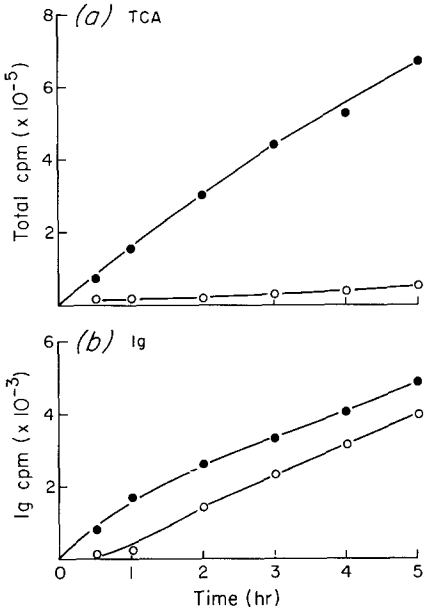

FIG. 5. Kinetics of incorporation of leucine-^{3}H into trichloroacetic acid–precipitable material and Ig of the bursa cells. Incorporation condition is the same as that used in the spleen cell suspension (Fig. 3). Intracellular protein, ●; secreted protein, ○; (*a*) trichloroacetic acid–precipitable material; (*b*) serologically precipitable Ig.

19S/7S ratio of 0.24 (Table II), the bursa cells apparently secrete Ig with a 19S/7S ratio of 0.36, as well as a significant amount of free L chain. After reduction and alkylation of secreted Ig, the H/L ratio of the spleen cells was 3.0 and that of the bursa cells 1.3, suggesting that the bursa cells secrete two times as many light chains per unit of Ig than do the spleen cells. A similar result is also observed with the intracellular Ig synthesized by these lymphoid cells (Table II). Fig. 7 shows a similar analysis of the intracellular Ig. As compared to the spleen cells which synthesize and secrete only 7S and 19S Ig, the bursa cells synthesize small amounts of Ig which migrate between 7S and 19S, as well as free L chains. No free H chains were detected inside the cell. The peak next to L chains was found to be L chain dimer by serology with anti-L chain anti-

serum. The absence of detectable free L chains in the cytoplasm and secreted material of the spleen cells suggests that the synthesis of H and L chains is apparently balanced in the spleen cells but not in the bursa cells (20). When

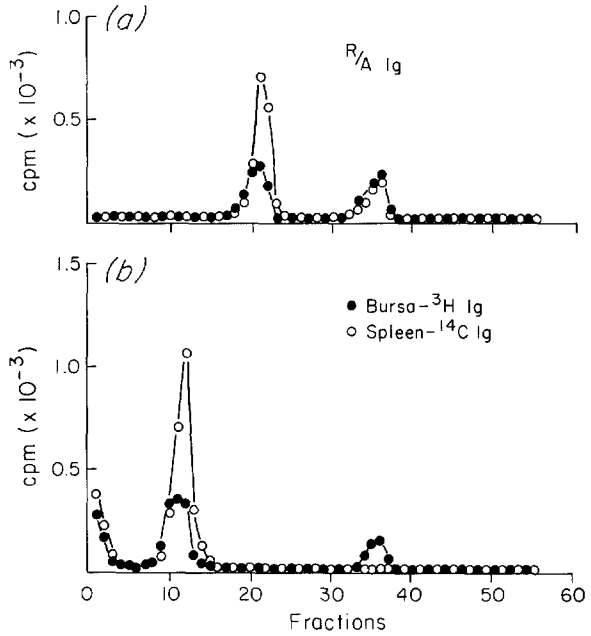

FIG. 6 Acrylamide gel electrophoresis of Ig secreted by the spleen and bursa cells. Leucine-^{3}H–labeled Ig secreted by the bursa cells was mixed in an adequate ^{3}H/^{14}C ratio with leucine-^{14}C–labeled Ig by the spleen cells, serologically precipitated with anti-IgM, and analyzed by SDS–acrylamide gel electrophoresis. ^{3}H-labeled bursa Ig, ●; ^{14}C-labeled spleen Ig, ○; (*a*) reduced and alkylated Ig; (*b*) total Ig.

TABLE II

Immunoglobulins Synthesized and Secreted by Chicken Lymphoid Cells

		cpm × 10⁻³			cpm × 10⁻³		H/L
		19S	7S	19S/7S	H	L	
Spleen	Secreted	0.6	2.5	0.24	0.9	0.3	3.0
	Intracellular	2.0	4.4	0.16	2.0	0.8	2.7
Bursa	Secreted	0.5	1.3	0.36	0.5	0.4	1.3
	Intracellular	1.0	3.5	0.28	3.2	2.0	1.6

serological precipitates were reduced and alkylated, the bursa Ig exhibited extra subunits of molecular size between H and L chains as seen by the three peaks in Fig. 7 *a*. This is not believed to be the result of incomplete reduction because the spleen intracellular Ig used as an internal standard were completely

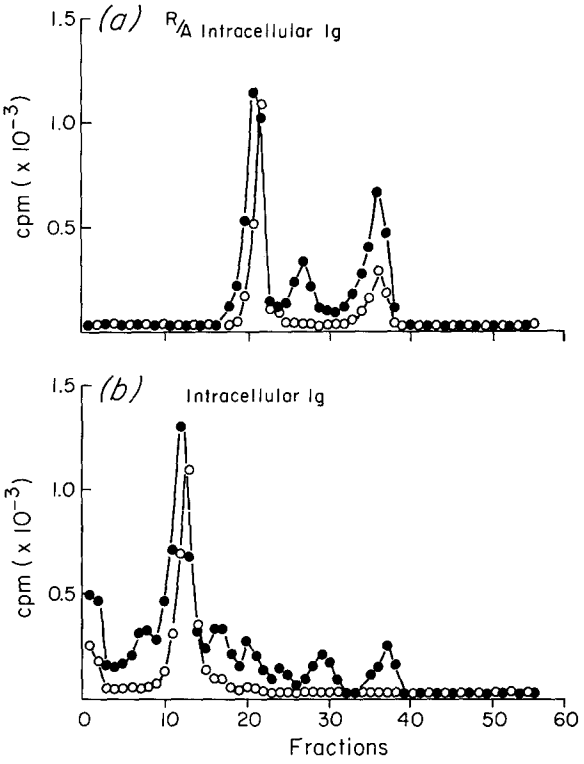

FIG. 7. Acrylamide gel electrophoresis of intracellular Ig synthesized by the spleen and bursa cells. Leucine-^{3}H–labeled bursa cell lysate was mixed with ^{14}C-labeled spleen cell lysate, serologically precipitated with anti-IgM, and analyzed by SDS–acrylamide gel electrophoresis. ^{3}H-labeled bursa Ig, ●; ^{14}C-labeled spleen Ig, ○; (*a*) reduced and alkylated Ig; (*b*) total intracellular Ig.

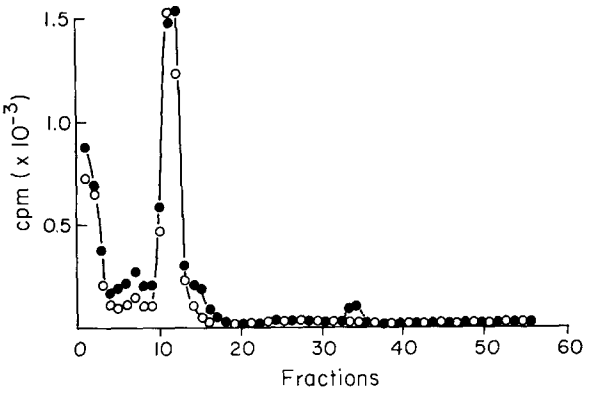

FIG. 8. Acrylamide gel electrophoresis of Ig synthesized and secreted by the spleen cells. Two batches of the spleen cells were separately labeled with leucine-^{3}H and -^{14}C for 3 hr. ^{3}H-labeled cell lysate was mixed with ^{14}C-labeled incubation media, serologically precipitated, and analyzed by SDS–acrylamide gel electrophoresis. ^{3}H-labeled intracellular Ig, ●; ^{14}C-labeled secreted Ig, ○.

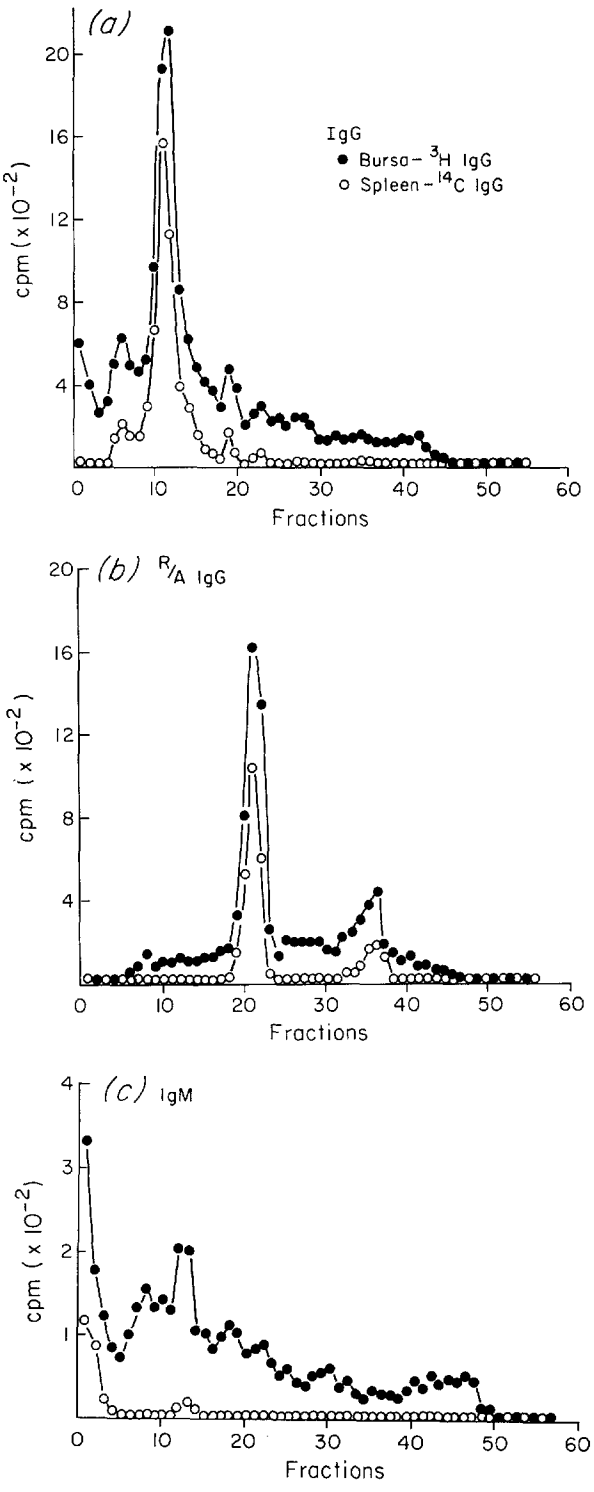
(a)
IgG
● Bursa – ^{3}H IgG
○ Spleen – ^{14}C IgG
cpm (x 10^{-2})
Fractions
(b) R/A IgG
cpm (x 10^{-2})
Fractions
(c) IgM
cpm (x 10^{-2})
Fractions

FIG. 9

reduced and alkylated. Furthermore, this component was not detected in Ig secreted by the bursa cells (Fig. 6 *a*). Such a structural difference in the Ig inside and outside the bursa cells was not observed in the spleen cells which exhibited an identical pattern of Ig, as shown in Fig. 8. In the spleen, both 19S (fraction No. 1–2) and 7S Ig (fraction No. 10–12) can be completely reduced and alkylated into H and L chain peaks without a sign of the third peak between them.

We looked for the origin of this middle peak by determining whether it is derived from IgM or IgG as described below. A batch of intracellular Ig of the bursa cells was labeled for 3 hr with leucine-^{3}H, serologically precipitated with anti-γ or anti-μ, and analyzed on SDS–acrylamide gel. Specificity of H chain specific antisera used in this experiment was monitored by introducing into the reaction mixture leucine-^{14}C–labeled Ig secreted by the spleen cells. Fig. 9 *a* shows acrylamide gel electrophoresis analysis of serological precipitates with anti-γ, which revealed only IgG at the 7S region and no significant amount of radioactivity on the top of the gel or in the region of L chains. When reduced and alkylated (Fig. 9 *b*), H and L chain peaks were produced with an H/L ratio of 2.3, which is almost identical to that of the spleen Ig (Table II). With anti-μ, we are now able to detect not only 19S IgM (gel fraction No. 1–2), but also 7S IgM (gel fraction No. 10–13) in acrylamide gel electrophoresis (Fig. 9 *c*). In contrast to the reduction product of IgG, these IgM peaks produced the third peak (i.e., H_0) migrating between H and L chains when fully reduced and alkylated (Fig. 9 *d*).

We tried to determine whether this middle peak of IgM is derived from H or L chains by estimating the H/L ratio. Since no free L chains were precipitated

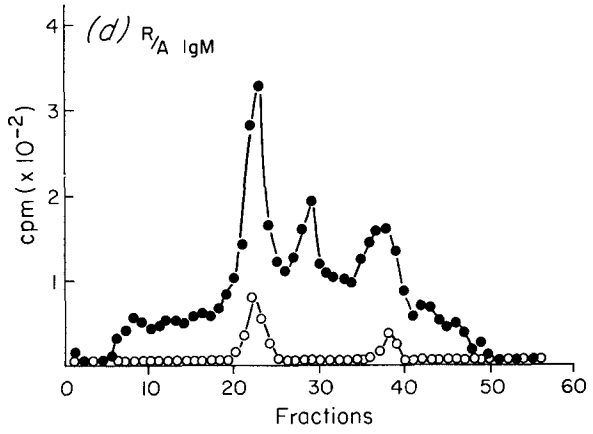

FIG. 9. Acrylamide gel electrophoresis of leucine-^{3}H–labeled intracellular Ig of the bursa cells. ^{14}C-labeled Ig secreted by the spleen cells was used as an internal control for serological specificity and acrylamide gel electrophoresis. ^{3}H-labeled bursa IgG, ●; ^{14}C-labeled spleen IgG, ○; (*a*) total IgG precipitated by anti-γ; (*b*) reduced and alkylated IgG; (*c*) total IgM precipitated by anti-μ; (*d*) reduced and alkylated IgM.

with these antisera, we can assume that all L chains recovered after reduction were derived from those bound to H chains in Ig. As summarized in Table III, the H/L ratio of the bursa IgG is 2.7, which is very close to the H/L ratio of the spleen Ig (Table II). In contrast to IgG, the H/L ratio of the bursa IgM is 1.5. The H + H_0/L ratio of the bursa IgM, is, however, 2.3, which is almost identical to that of the bursa IgG or spleen Ig. From this calculation, we conclude that this middle peak, H_0 component with molecular weight of 50,000, is related to μ-chains rather than to L chains. At the present moment, we did not determine whether it is derived from 7S or 19S IgM.

A similar incorporation experiment with the thymus cells failed to show any significant radioactivities serologically precipitable with anti-IgM. No evidence of H and L chain synthesis was detected in the thymus by acrylamide gel electrophoresis of the serological precipitates.

TABLE III

*Subunits of Immunoglobulins Synthesized by the Bursa Cells**

	cpm × 10^{-3}				
	H	H_0	L	H/L	H + H_0/L
Anti-μ	2.0	1.5	1.3	1.5	2.3
Anti-γ	4.4	0	1.6	2.7	2.7

* Prepared from the data of Fig. 9.

DISCUSSION

Biosynthesis and secretion of Ig by normal lymphoid cells was studied in the chicken, which has anatomically distinct lymphoid tissues (1, 21). From the observations made in this study the following conclusions may be drawn. (*a*) The majority of the proteins secreted by the spleen cells (i.e., more than 70%) were serologically precipitable Ig (Fig. 4 *b*); (*b*) the relative amount of Ig produced by the spleen cells varies depending upon the state of immunization, while immunization does not affect the capacity of the bursa cells to synthesize Ig (Table I); (*c*) a population of the bursa cells apparently accumulates Ig inside the cells which may not be secreted (Fig. 5); (*d*) the Ig which is not secreted from the bursa cells is shown to be IgM by serological precipitation and SDS–acrylamide gel electrophoresis (Fig. 7); (*e*) H chains of this IgM appear to be faster-migrating H_0 chains which lie between H and L chains (Fig. 9).

We came to the conclusion that the subunit (H_0) is a faster-migrating H chain than the L chain, based upon the result of calculating the H/L ratio of IgM precipitated by anti-μ serum (Fig. 9 and Table III). It is possible that the difference in migration in acrylamide gel electrophoresis between two H chains may be consequent to a lack of carbohydrate residues rather than to significant differences in protein size (22). H_0 chains may have less protein-bound carbo-

hydrate than H chains of IgM or IgG. The carbohydrate composition of these H chains is currently being examined. The presence of two forms of intracellular heavy chain with different carbohydrate compositions has been reported in IgM producing mouse myeloma cells (16, 22). Ig which were precipitable with anti-μ serum and which migrated between 8S and 19S have been reported in malignant human lymphocytes (23). 7S IgM may be the intracellular precursor of the fully assembled 19S protein before secretion (24). We doubt, however, that 7S IgM which is composed of H_0 and L chains is also such a precursor because the H_0 chain was not detected in the secreted proteins when fully reduced. It appears to us that the bursa may contain three different clones of lymphoid cells synthesizing Ig: one with nonsecretory IgM, the second with 19S IgM, and the third with 7S IgG. During development of lymphoid cells in the bursa it was shown that IgG-producing cells arise from a clone of IgM-producing cells (25, 26). We rather believe that IgM containing H_0 chains as H chains are synthesized by the clone of the cells which may be the precursor of 19S IgM–producing cells. It could even be suggested that the cells synthesizing nonsecretory Ig may represent a precursor common to both lymphoid cells secreting IgM or IgG. The previous studies by Kincade et al. (26) could not distinguish between IgM with H and IgM with H_0. The ontogenetic relation among the three clones mentioned above will be further studied by analyzing the relative proportion of the two kinds of H chains of IgM at various ages of chick embryo.[2]

Nonsecretory Ig has been reported in Burkitt lymphoma cell lines (27, 28). It has been previously suggested by Klein et al. (27) that these malignant cell lines may have arisen from normal lymphocytes which do not secrete Ig. Our experiments clearly indicate that such cell lines may be derived from bursa-dependent or bone marrow–dependent B cells which are the precursors of plasma cells secreting Ig rather than thymus-dependent T cells (1, 29). Among several classes of Ig detected on the surface of antigen-binding cells, μ-chains are reported to be the predominant H chains (29–34). We found that, when the chicken was sensitized with BGG, the bursa cells do not synthesize antibody-binding antigen while the spleen cells in the same chicken synthesize and secrete 19S IgM and 7S IgG antibodies.[3]

SUMMARY

Synthesis and secretion of Ig by chicken lymphoid cells was studied. Both spleen and bursa cells synthesize and secrete IgM and IgG whereas Ig was not detected in thymus cells. In contrast to the spleen cells which synthesize H and L chains in balanced quantities, the bursa cells synthesize and secrete free L chains. In addition to the lymphoid cells which secrete IgM or IgG, the bursa appears to contain a cell population which synthesizes nonsecretory Ig. The

[2] Choi, Y. S., and R. A. Good. Manuscript in preparation.

[3] Choi, Y. S., and R. A. Good. Manuscript in preparation.

structure of this Ig was studied by specific serological precipitation and by SDS–acrylamide gel electrophoresis. The H chains of this nonsecretory Ig are serologically related to μ-chains and exhibit a smaller molecular weight (i.e., ~50,000) in SDS–acrylamide gel electrophoresis than H chains of IgG and IgM synthesized by the spleen cells (i.e., ~70,000).

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