Biosynthesis of Membrane Bound Ig and Secretion of Ig by Chicken Lymphoid Cells*

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ABSTRACT: The metabolic turnover of membrane proteins of chicken lymphoid cells is studied, using a double isotope labeling technique (i.e., [^{14}C]amino acid pulse and [^{3}H]leucine chase). Compared with other membrane proteins, the metabolic turnover of membrane bound immunoglobulins (M-Ig) is very slow. There was no difference in the turnover between M-Ig and specific antigen binding receptor immunoglobulins. Immunoglobulins appear to be a stable constituent of the lymphocyte membrane. Cellular kinetic experiments show that the rate of biosynthesis of secreted immunoglobulins (S-Ig) is nearly ten times as much as that of M-Ig, suggesting that metabolic pathways leading to M-Ig are distinct from those leading to S-Ig. The difference in ^{3}H/^{14}C ratios between S-Ig and M-Ig reflects the rate of biosynthesis of these immunoglobulins by two types of bursa derived lymphoid cells.

It is now known that specific binding of antigen to the "receptors" of bursa derived lymphoid cells (B cells) causes antigen binding cells to differentiate into antibody secreting cells (Nordin et al., 1970; Davie and Paul, 1971). Immunoglobulins (Ig[1]) on the surface of B cells, detected by the surface radioiodination method (Greaves and Hogg, 1971; Vitetta et al., 1971; Marchalonis et al., 1972) or by competitive inhibition of antigen binding by anti-Ig sera (Davie and Paul, 1971; Wigzell, 1970), have been assumed to be the receptor, although direct evidence demonstrating receptor molecules is lacking (Warner, 1974). In order to specify the receptor, we have now studied metabolic turnover of membrane bound Ig (M-Ig) and antigen binding receptors,[2] in comparison with that of secreted Ig (S-Ig). This paper shows that, unlike other membrane proteins, the metabolic turnover of M-Ig is very slow, suggesting that M-Ig is a stable constituent in the lymphocyte membrane and that metabolic pathways leading to M-Ig are distinct from S-Ig.

Experimental Section

Chicken Lymphoid Cells. White Leghorn chickens (Line 96, Hy-Line Poultry Farm, Johnston, Iowa), 12–15 weeks old, were immunized by intravenous injection of 20 mg of bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) 5 days prior to experimentation. Suspensions of lymphoid cells from chicken spleens were prepared as described previously (Choi and Good, 1972). Lymphoid cells were further purified by Ficoll-Hypague gradient centrifugation (Thorsby and Bratlie, 1970) to remove granulocytes and erythrocytes, washed three times with RPMI 1640, and suspended in an appropriate medium. Lymphoid cells thus purified appeared morphologically homogeneous.

Fractionation of Membranes. In view of electron microscopic evidence that chicken lymphoid cells consist of a large nuclei surrounded by a thin layer of cytoplasm (Clawson et al., 1967), it is very difficult to break the cells efficiently with commonly used glass homogenizers. Cytoplasmic membranes were prepared accordingly by the method described below, which is a modification of methods widely used for isolating plasma membranes from other tissue cells (Warren et al., 1966; Kiehn and Holland, 1970).

Lymphoid cells were swollen (10^7 cells/ml) in 0.05 M Tris-HCl (pH 7.4) with 0.005 M $MgCl_2$ and homogenized vigorously (50–60 strokes) in an ice–water bath, using a Dounce homogenizer with a tightly fitting stainless steel pestle (clearance 0.002 in.) (Choi et al., 1971a). The homogenate was made 10% with respect to sucrose and layered on a discontinuous sucrose gradient made of equal volumes of 30% (w/v) and 50% sucrose containing 0.005 M $MgCl_2$. All procedures were performed at 0–4 °C. Centrifugation (1400*g* for 15 min) sedimented nuclei, mitochondria, and unbroken cells. The fluffy material at the interface between the 10 and 30% layers, and extending into the 30% sucrose, was collected and pelleted by centrifugation at 9000*g* for 10 min. This membrane pellet was further fractionated by gentle resuspension in 3 ml of 30% sucrose and layered over a discontinuous sucrose gradient made of equal volumes (3 ml) of 40, 45, and 55% (w/v) sucrose and centrifuged at 200 000*g* for 100 min. At the end, three turbid layers were found at the interfaces of the sucrose gradient. These membrane fractions were termed I, II, and III, reading from the top (light) and to bottom (heavy) of the tube. These fractions were collected separately, washed in 0.05 M tris(hydroxymethyl)aminomethane-HCl (pH 7.6, 4 °C)–0.025 M KCl–0.005 M $MgCl_2$ (TKM) using a high-speed centrifuge, and used for subsequent analysis of immunoglobulins and other proteins.

Enzyme Assays. 5′-Nucleotidase activity was assayed by the methods described by Heppel and Hilmoe (1951); inorganic phosphate for this enzyme assay was determined by the method of Fiske and Subbarow (1925). Succinate cytochrome *c* reductase activity was measured by the method of Nason and Vasington (1963).

Determination of RNA and Protein. Separation of RNA

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[1] Abbreviations: BSA, bovine serum albumin; Ig, immunoglobulin; M-Ig, membrane bound Ig; NP-40, Nonidet-P 40; PBS, 0.02 M phosphate buffer (pH 8.0)–0.14 M NaCl; S-Ig, secreted Ig; TCA, trichloroacetic acid; TKM, 0.05 M tris(hydroxymethyl)aminomethane-HCl (pH 7.6, 4 °C)–0.025 M KCl–0.005 M $MgCl_2$.

[2] Antigen binding Ig solubilized from plasma membranes.

and protein was done by the method of Shibko et al. (1967). The amount of RNA was measured by the method of Munro and Fleck (1966) and protein by the Lowry method, using bovine serum albumin (Sigma Chemical Co.) as standard (Lowry et al., 1951).

Incorporation of Radioisotopes. To label cellular proteins with ^3H or ^{14}C (Choi and Good, 1972), lymphoid cells were suspended in Eagle's media containing 5% decomplemented horse serum and 1/20 the normal amounts of leucine at a density of 5×10^7 cells/ml and were then incubated in a spin bottle at 37 °C after addition of 5–10 μCi/ml of L-[4,5-^3H]leucine (50–60 Ci/mmol, Schwartz BioResearch, Inc., New York). The humid atmosphere contained 15% CO_2–85% air. Eight to twelve hours later, cells were collected by centrifugation and membrane fractions isolated.

Double Labeling the Membranes. Uniformly labeled L-[^{14}C]leucine (285 mCi/mM), L-[^{14}C]threonine (205 mCi/mM), and L-[^{14}C]valine (244 mCi/mM) were obtained from New England Nuclear. For double-labeling experiments, the cells were first incubated in the media (containing 5% decomplemented horse serum and 1/20 the normal amounts of L-leucine, L-threonine, and L-valine) with [^{14}C]amino acids (3 μCi/ml) for 6–7 h to label the membrane proteins with ^{14}C. The cells were then collected by centrifugation, suspended in incorporation medium containing [^3H]leucine, and incubated for an additional 12–15 h. Conditions of culture for [^3H]leucine incorporation were essentially the same as described above. Under these conditions, we found that the total radioactivity incorporated, per 10^6 cells, was proportional to the product of the cell and radioisotope concentrations. Since it is practically impossible to obtain sufficient ^{14}C-labeled Ig by using [^{14}C]leucine alone, we used three [^{14}C]amino acids to increase ^{14}C-specific radioactivity of radiolabeled Ig. Relative concentrations of ^3H and ^{14}C were chosen so that the ratio of $^3H/^{14}C$ incorporated into proteins would not exceed 5:1. After 20–24 h incubation, the number of viable cells recovered was 70–80% that of the starting cell suspensions.

Cell Surface Labeling with ^{125}I. The cell surface was labeled with ^{125}I using lactoperoxidase (Marchalonis, 1972; Phillips and Morrison, 1970); 2×10^7 cells were suspended in 50 μl of 0.05 M phosphate buffer (pH 7.2)–0.1 M NaCl containing 14 μg of lactoperoxidase and 2 mCi carrier-free Na^{125}I (Amersham-Searle, Inc.), and the reaction was started by adding 10 μl of 0.03% H_2O_2 at 30 °C. After 10 min incubation, the cells were washed twice with cold PBI (0.05 M phosphate buffer (pH 7.2)–0.1 M NaI) and mixed with 10^9 cold cells, and membrane fractions were prepared.

Serological Precipitation of Chicken Immunoglobulins. Radiolabeled Ig bound to the membranes was quantitatively assayed by serological precipitation with specific anti-chicken Ig antisera (Choi and Good, 1972). The pellets of membrane fractions were suspended in 1.0 ml of TKM, solubilized by 1% Nonidet-P 40 (NP-40) (Shell Chemical Co.), and centrifuged 150 000*g* for 60 min to sediment ribosomes. Radioactive Ig in the supernatant was complexed with an excess of rabbit antichicken Ig serum, and the complexes were precipitated by goat antirabbit IgG. The detailed method of preparation of serological precipitates for quantifying radioactive chicken Ig has been described previously (Choi and Good, 1972).

Polyacrylamide Gel Analysis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of membrane proteins was performed in a sulfate–borate discontinuous buffer system as described by Neville and Glossmann (1971). The membrane pellet was solubilized in 2% sodium dodecyl sulfate–0.02% Na_2CO_3–5% dithiothreitol and heated at 60 °C for 1 min, and then less than 200 μg of proteins was applied to an 11.5% gel (0.6×10 cm^2).

At the end of electrophoresis, the gels were fractionated using a Gelman slicer and the radioactive Ig was measured, as previously described (Choi and Good, 1972). Preparation of serological precipitates for sodium dodecyl sulfate–polyacrylamide gel electrophoresis and counting of fractionated radioactivity have already been described elsewhere (Choi et al., 1971a).

Isolation of Antigen Binding Receptors by Immune Adsorption. For immune adsorption, radiolabeled ($^3H/^{14}C$) membrane proteins which had been solubilized by 1% NP-40–TKM were mixed with 0.2 ml of BSA–arm-Sepharose (25 mg of BSA/ml) in the presence of 0.1% horse serum and incubated at 37 °C for 60 min and at 4 °C overnight. The beads were centrifuged down and washed exhaustive with 0.5% NP-40–PBS until radioactivity could no longer be detected in the washings. Adsorbed $^3H/^{14}C$-labeled proteins were eluted from BSA–arm-Sepharose by 0.5 ml of 2 M KSCN (Choi and Good, 1973). Two consecutive elutions were pooled together and dialyzed against 0.05 M phosphate buffer (pH 7.6) and lyophilized, both for acrylamide gel electrophoresis and for measuring radioactivity. The first two elutions usually accounted for more than 80% of adsorbed radioactivity.

To isolate secreted antibodies, an aliquot of the incubation medium collected at the end of the incorporation experiment was exhaustively dialyzed against PBS to remove free [^3H]leucine and then subjected to immune adsorption.

Preparation of BSA–arm-Sepharose. Bovine serum albumin (Sigma Chemical Co.) was covalently attached to the aminoalkylagarose by the method described by Cuatrecasas and Parikh (1972). The aminoalkylagarose was prepared by treating 50 ml (packed volume) of cyanogen bromide activated Sepharose 4B with 100 μmol of 3,3′-diaminodipropylamine at pH 10 and mixed overnight in the cold room. The beads were then exhaustively washed with distilled water and mixed with 50 mmol of succinic anhydride at pH 6 for 5 h at 4 °C and washed again with distilled water. The washed, succinylated agarose was extensively treated with dioxane to remove water and suspended in dioxane (three times the volume of the packed Sepharose). To activate this Sepharose derivative, solid *N*-hydroxysuccinimide and *N,N′*-dicyclohexylcarbodiimide were added to their respective concentrations of 0.1 M. The mixture was gently stirred for 70 min at room temperature. The activated Sepharose was washed over a Büchner funnel with six volumes of dioxane, followed by six volumes of methanol, and then by further washings with another six volumes of dioxane. The beads were kept suspended in anhydrous dioxane.

To attach BSA to the activated Sepharose ester derivative, salt-free lyophilized BSA (2.0 g) was dissolved in 50 ml of 0.1 M phosphate buffer (pH 7.0), mixed with 50 ml of activated Sepharose ester derivative, and tumbled in the glass bottle wrapped in tin foil for 2 h in the cold room. By this time, the amount of attached BSA reached a plateau. Solid glycine was added to the mixture to the concentration of 1 M to mask the remaining reactive sites, and the mixture was tumbled overnight in the cold room. The beads were then packed into a small column, mixed with 200 ml of 2 M KSCN, and exhaustively washed with PBS. With this method we prepared BSA-arm-Sepharose (25 mg/ml).

Table I: Enzymes in the Membrane Fractions of Chicken Lymphoid Cells.

	Specific Activity				
	H	N	I	II	III[c]
5′-Nucleotidase[a]	1.2	0.41	14	14	4.1
Succinate cytochrome *c* reductase[b]	0.6	0	0	0	2.6

[a] Specific activity = mmol of Pi formed/mg of protein min^{-1}. [b] Specific activity = mmol/mg of protein min^{-1}. [c] H, homogenate; N, nuclear pellet; I, II, III, membrane fractions.

Results

Membrane Fractions. Membrane fractions of chicken lymphoid cells formed three layers in a discontinuous sucrose gradient at interfaces with densities of 1.15 (I), 1.17 (II), and 1.19 (III). In order to distinguish which fraction contained plasma membrane, four methods were employed to characterize the sucrose gradient fractions: direct chemical determination, cell surface labeling with ^{125}I by lactoperoxidase, enzyme analysis, and electron microscopy.

RNA and protein profiles across the sucrose gradient of a typical run are shown in Figures 1a and 1b. The major portions of proteins and RNA were recovered in the heaviest membrane fractions (III), suggesting that this fraction contained membrane bound and free polysomes at which membrane proteins and Ig are synthesized (Choi et al., 1971b; Jamieson and Palade, 1967). Examination of each gradient fraction by electron microscopy (performed by Dr. E. de Harven at the Memorial Sloan-Kettering Cancer Center, New York, N.Y.) also showed that fraction III contained far more polysomes than fraction I, supporting the results of chemical analysis. Electron microscopy of fractions I and II revealed smooth membrane structures and some ribosomes.

It has already been shown that the surface proteins of intact cells are covalently labeled with ^{125}I by lactoperoxidase (Marchalonis et al., 1972; Phillips and Morrison, 1970). Hence, proteins labeled by this method can be expected to concentrate in the fraction from a sucrose gradient that contains plasma membrane. Intact lymphoid cells were labeled with ^{125}I by lactoperoxidase, fractionated as described above, and the profile of TCA precipitable radioactivity in the sucrose gradient was determined. As shown in Figure 1c, the highest specific radioactivity recovered was in fractions I and II.

Further characterization of membrane fractions was attempted by analysis of marker enzymes. 5′-Nucleotidase is well known to be a marker enzyme for plasma membrane, while mitochondria can be detected by assaying for succinate dehydrogenase (Evans, 1970; Touster et al., 1970; Zimmerman, 1974). Table I shows the specific activities of these two enzymes measured in the different membrane fractions. Fractions I and II revealed the highest specific activity of 5′-nucleotidase, more than tenfold that of a crude homogenate. Succinate dehydrogenase was not detectable in these fractions, but a significant amount was found in fraction III. Enzyme analysis, in addition to localization of [^{125}I]labeled proteins, suggests that the membrane fractions I and II of chicken lymphoid cells appeared markedly enriched in plasma membranes when compared with the original homogenate, but electron microscopy revealed that these fractions also contained some ribosomes.

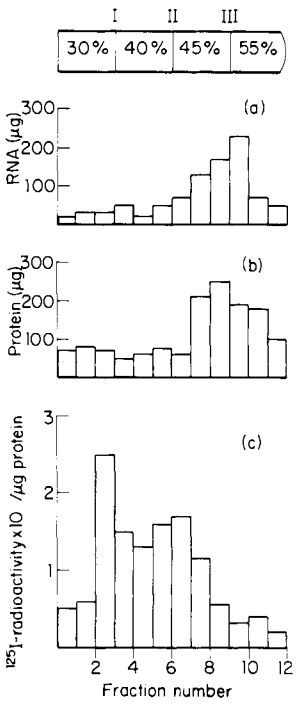

FIGURE 1: Distribution of RNA, protein, and surface labeled ^{125}I in the membrane fractions of chicken lymphoid cells. Lymphoid cells (4 × 10^9) were purified by Ficoll-Hypaque centrifugation from chicken spleens. A postnuclear supernatant (3 ml) was layered on a 9-ml discontinuous sucrose gradient and centrifuged at 200 000*g* for 100 min. At the end of centrifugation, 1-ml fractions were collected from the top of the gradient. RNA and proteins in each fraction were determined as described in Methods. For measuring distribution of surface labeled ^{125}I, 2 × 10^7 cells were labeled with ^{125}I by lactoperoxidase, washed with PBS, and mixed with 10^9 unlabeled cells. Membranes were fractionated by the method described above. Specific radioactivity (cpm/μg protein) was calculated by measuring radioactivity and protein concentration in each fraction: (a) RNA; (b) protein; (c) ^{125}I specific activity.

The experiment described below further established that fractions I and II clearly differ from III.

Metabolic Turnover of the Membrane Proteins. We have employed a double isotope technique (i.e., [^{14}C]amino acid pulse and [^{3}H]leucine chase), as described by Arias et al. (1969) for measuring the relative turnover rates of the membrane proteins and membrane bound Ig. For any one protein, the level of ^{3}H radioactivity indicates new synthesis plus metabolic decay, whereas ^{14}C radioactivity indicates the amount of metabolic decay. The amount of metabolic decay should be determined by the rate of intracellular degradation and of secretion. Proteins that are synthesized and rapidly degraded or secreted will have high ^{3}H/^{14}C ratios. The assumptions and validity of this method were extensively discussed by Arias et al. (1969), as well as experimentally proved by Dehlinger and Schimke (1971).

Figure 2 shows sodium dodecyl sulfate–acrylamide gel electrophoresis of detergent solubilized membrane fractions. A marked heterogeneity in the ratios of ^{3}H/^{14}C among the protein constituents reflects heterogeneity in their metabolic turnover. There are some differences in the turnover rates of the individual membrane proteins between fractions I and III. However, it appears that the overall turnover rate of a more internal membrane (III) is slightly greater than that of the plasma membrane protein (I). In particular, there are definite decreases in ^{3}H/^{14}C ratios where the positions of protein in sodium dodecyl sulfate–acrylamide gel electrophoresis correspond to the molecular

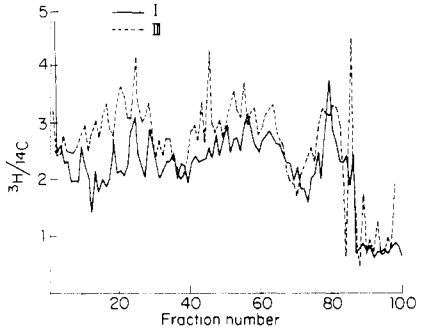

FIGURE 2: Metabolic turnover of membrane proteins. $^3H/^{14}C$ ratios were calculated from each fraction in sodium dodecyl sulfate–acrylamide gel electrophoresis of $^3H/^{14}C$ labeled membrane fractions in a sulfate–borate discontinuous buffer system: (—) I; (- - -) III.

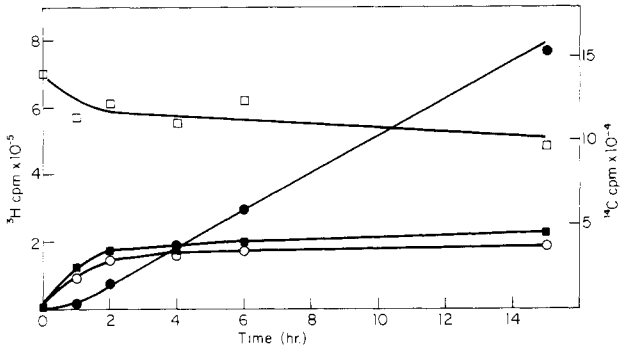

FIGURE 3: Kinetics of incorporation of [^{3}H]leucine into intracellular and secreted Ig of chicken lymphoid cells. A cell suspension (5×10^6 cells/ml) was first incubated with [^{14}C]leucine, -threonine, and -valine for pulse labeling intracellular Ig, pelleted by centrifugation and suspended in the media containing [^{3}H]leucine. Aliquots of 3 ml were distributed in petri dishes. At each time point, a cell suspension was transferred from a petri dish to a glass tube. The cells were separated from the incubation media by centrifugation. Radiolabeled intracellular Ig and secreted Ig in the media were determined as described in Methods: (●) [^{3}H]S-Ig; (■) ^{3}H-intracellular Ig; (○), [^{14}C]S-Ig; (□), ^{14}C-intracellular Ig.

sizes of H (fraction 36–38) and L chains (fraction 70–72) of chicken Ig.

Biosynthesis of Membrane Bound Ig and Secretion of Ig. The kinetics of synthesis and secretion of Ig were studied by incubating a ^{14}C-labeled cell suspension with [^{3}H]leucine, following the ^{3}H-labeling of Ig.

As shown in Figure 3, the amount of ^{3}H-labeled Ig inside the cell increased rapidly for about 3 h and then reached a plateau. It should be noticed, however, that there is persistent and slow increase in the plateau level, indicating a slight expansion of the intracellular pool. The rate of secretion of ^{3}H-labeled Ig increased after a lag of 1 h and became constant throughout 14 h of incubation.

During this period, a portion (i.e., ~30%) of the Ig prelabeled with ^{14}C secreted (chased) out of the cell without an initial lag; secretion stopped after 4 h. At the same time, the intracellular content of ^{14}C-labeled Ig decreased rapidly for about 3 h and then very slowly thereafter. Under the experimental conditions where the sum of secreted and intracellular ^{14}C-labeled Ig remained the same, synthesis and secretion of ^{3}H-labeled Ig took place at normal rates (Choi and Good, 1972). More than 80% of the radioactive proteins secreted were serologically precipitable Ig.

The difference in kinetics of Ig secretion and of M-Ig synthesis is more remarkable if we plot the changes in $^3H/^{14}C$ ratios of Ig. As shown in Figure 4, the rate of increase

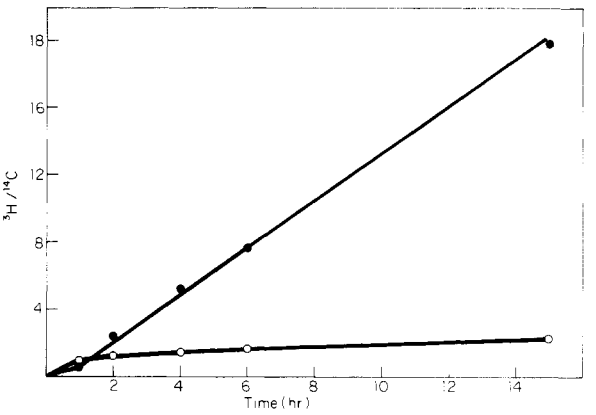

FIGURE 4: Changes in the ratio of $^3H/^{14}C$ in intracellular and secreted Ig. The ratios were calculated for each sample time from Figure 3: (○) intracellular Ig; (●) secreted Ig.

Table II: Metabolic Turnover of M-Ig and S-Ig.

	M-Ig	Antigen binding Ig	Ab–Ig/Ig[a]	S-Ig	Specific Antibodies	Ab/Ig[b]
^3H	51 720	13 780	27%	67 000	15 700	23%
^{14}C	28 890	6 040	21%	8 550	2 020	24%
$^3H/^{14}C$	1.8	2.3		7.8	7.8	

[a] Ab-Ig, antigen binding Ig. [b] Ab, antibody.

in $^3H/^{14}C$ of secreted Ig is nearly ten times as much as that of intracellular Ig, suggesting a distinct metabolic pathway for S-Ig and M-Ig.

Metabolic Turnover of Membrane Bound Ig (M-Ig). The turnover of M-Ig was further analyzed with purified membrane fractions (Table II). Under the experimental conditions used, $^3H/^{14}C$ ratios of M-Ig were 1.8, while $^3H/^{14}C$ ratios of S-Ig were 7.8 (Table II).

We also separated antigen binding receptors[2] from other membrane proteins by solubilizing the membrane fractions with a nonionic detergent, NP-40, and specific immune adsorption to insolubilized antigen (i.e., BSA-arm-Sepharose). Antigen binding proteins isolated by this method have already been shown to be specific (Choi and Good, 1973). In the same radiolabeled membranes (Table II), 27% of the M-Ig was capable of binding specific antigen, BSA, and the turnover of antigen binding receptors was the same as that of M-Ig. At the same time, $^3H/^{14}C$ ratio of S-Ig was 7.8, and 23% of the S-Ig was specific antibodies with the same $^3H/^{14}C$ ratio.

Since $^3H/^{14}C$ ratios of S-Ig simply represent a cumulation of secreted ^{3}H-Ig over a constant amount of [^{14}C]Ig, the difference in $^3H/^{14}C$ ratios will increase as the incubation period is prolonged (Figure 4).

Metabolic Turnover of H and L Chains of M-Ig. The turnover rates of H and L chains of M-Ig were studied by sodium dodecyl sulfate–acrylamide gel electrophoresis analysis of serological precipitates (Figure 5). The radioactivity calculated under H- and L-chain peaks is summarized in Table III. With regard to Ig subunits, the metabolic turnover rate of H chains is twice that of L chains, suggesting that Ig L chains appear to have a longer lifetime within the cell than H chains. At the same time, the H/L chain ratio of [^{3}H]Ig is twice that of [^{14}C]Ig. Since there is no evidence of free ^{14}C L chains (Figure 5), the low ratio of H/L of ^{14}C

Table III: Metabolic Turnover of Membrane Bound Ig.

		^3H	^{14}C	$^3H/^{14}C$
I	H	2300	820	2.8
	L	735	550	1.3
	H/L	3.13	1.50	
II	H	2570	1070	2.4
	L	765	690	1.1
	H/L	3.36	1.60	
III	H	7930	2900	2.7
	L	2610	1760	1.5
	H/L	3.04	1.66	

radioactivity suggests the presence of Ig molecules composed of ^3H H chains and ^{14}C L chains.

Discussion

M-Ig as a Protein Constituent of Membranes. Biochemical characterization of the protein constituents of the plasma membrane of lymphoid cells has been performed in several laboratories: pig (Allan and Crumpton, 1971); rabbit (Schmidt-Ullrich et al., 1974); chicken (Ragland et al., 1973); and human lymphocytes (Lopes et al., 1973).

The biosynthesis and degradation of membrane proteins have been studied using a double isotope technique: [^{14}C]amino acid pulse and [^3H]leucine chase (Arias et al., 1969). With the membrane fractions separated from chicken lymphoid cells, we found a marked heterogeneity in the turnover of the membrane protein constituents (Figure 2), as has been reported for the membrane proteins of rat liver (Dehlinger and Schimke, 1971; Omura et al., 1967; Bock et al., 1971).

It has been suggested by Palade (1959) that the plasma membranes of eukaryotic cells are not synthesized de novo but rather formed from intracellular cytoplasmic membranes by a membrane fusion process. By analogy with the mechanism by which protein molecules are secreted by pancreatic cells, the membrane proteins of the lymphoid cell are synthesized in polysomes, incorporated into rough endoplasmic reticulum, transmitted and then converted into the plasma membrane (Singer, 1974). During the process of intracellular transport and membrane fusion, some protein components may be inserted or deleted. Heterogeneous turnover rates of protein constituents in each membrane fraction suggest that the membrane is in a continuous state of flux, in which individual proteins are inserted and deleted at different rates. This would account for differences in sodium dodecyl sulfate-acrylamide gel electrophoresis profiles of the membrane proteins in fractions I and III (Figure 2). Since protein is synthesized in rough endoplasmic reticulum, metabolic degradation should take place during transport from the rough endoplasmic reticulum to the plasma membrane. Our experimental results show that the turnover rate of M-Ig is the same regardless of whether it is isolated from the plasma membrane or intracellular cytoplasmic membrane (Table III). The identical turnover rates for each protein constituent suggest that such degradation takes place after the protein molecules dissociate from the membranes. Therefore, the rates of biosynthesis and degradation of an identifiable protein, M-Ig, directly support this assembly line concept (Singer, 1974).

Synthetic Pathways Leading to M-Ig and S-Ig. Cellular kinetic experiments (Figure 4) showed that the rates of increases in $^3H/^{14}C$ ratios of S-Ig were far greater than M-Ig, indicating that synthetic pathways of M-Ig are different from S-Ig.[3] The remarkable difference in $^3H/^{14}C$ ratios between S-Ig and M-Ig simply reflects the rates of biosynthesis of these Ig's by two types of B cells: i.e., "plasma cell-like" B cells which synthesize and secrete Ig at the rate of at least eight- to tenfold greater than small B cells. Andersson et al. (1974) showed that, in mouse lymphoid cells, large cells synthesize and secrete 19S IgM while small cells synthesize and shed 7S IgM in much slower rates than large cells, thus supporting the above interpretation.

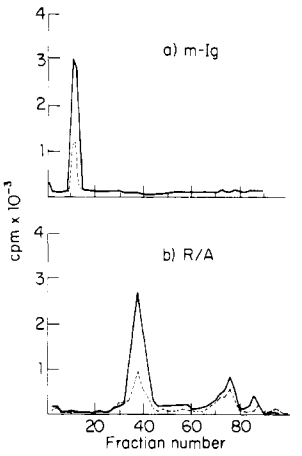

FIGURE 5: Sodium dodecyl sulfate-acrylamide gel electrophoresis of $^3H/^{14}C$-labeled chicken Ig. $^3H/^{14}C$-labeled membrane fraction I was solubilized by NP-40 and centrifuged 105 000*g* for 60 min. Specific serological precipitates on the supernatant with rabbit antichicken-Ig were washed with PBS and analyzed in sodium dodecyl sulfate-acrylamide gel electrophoresis in a sulfate-borate discontinuous buffer: (a) nonreduced; (b) reduced and alkylated; (—) ^3H; (- - -) ^{14}C.

The $^3H/^{14}C$ ratios of M-Ig obtained from membrane fractions are also fourfold less than $^3H/^{14}C$ ratios of S-Ig (Table II), which is consistent with the results obtained with whole cells. These results suggest that M-Ig is not an immediate precursor of S-Ig in transit through the plasma membrane and exclude the possibility that M-Ig may be secreted Ig which has become bound or adsorbed to the plasma membrane after secretion (Knopf, 1973).

With mouse myeloma cells (Choi et al., 1971a,b), it was previously shown that Ig was synthesized on membrane bound polysomes, incorporated into rough endoplasmic reticulum, transported from rough endoplasmic reticulum to the plasma membrane, and then secreted. Therefore, it was expected that almost all the ^{14}C-labeled secretory Ig would be transported out of the cell during the [^3H]leucine chase period (i.e., 4–6 h). However, under the experimental conditions where synthesis and secretion of [^3H]Ig took place at a linear rate, 70% of [^{14}C]Ig remained inside the cell. At the same time, we did not find evidence of rapid degradation of M-Ig since the sum of intracellular [^{14}C]Ig and secreted [^{14}C]Ig remained the same during 14 h of the ^3H-chase period (Figure 3). M-Ig appears to be metabolically stable, as shown by its low $^3H/^{14}C$ ratio. Andersson et al. (1974) found that the half-life of 19S IgM, synthesized by mouse B cells, was 4 h while that of 7S IgM was 20 to 80 h.

[3] Since secreted antibodies are known to be identical, in regard to specificity and affinity to antigen, with M-Ig of antigen binding B lymphocytes (Davie and Paul, 1971), it is assumed that overall amino acid composition of S-Ig may be a little different from that of M-Ig. Hence, it is not likely that the apparent differences in $^3H/^{14}C$ ratios may result from differences in relative contents of threonine or valine between M-Ig and S-Ig.

Receptor Ig as an M-Ig. Antigen binding proteins were isolated from lymphoid cell membranes by specific immunoadsorption. It was shown that these proteins may be the receptors of B lymphoid cells because (1) they bind only specific antigen (i.e., BSA); (2) antigen binding is competitively inhibited by specific antigen; (3) they are synthesized by lymphoid cells prepared from immunized chickens and not from unimmunized chickens; and (4) more than 90% eluted radioactivity were identified as Ig by serological precipitation with anti-Ig and sodium dodecyl sulfate-acrylamide gel electrophoresis (manuscript in preparation).

It has also been suggested that the receptors isolated 5 days after intravenous immunization are synthesized by bursa derived (B) cells. At this time, the number of antigen binding cells reached their peak (Kiszkiss et al., 1972). Furthermore, such antigen binding cells could not be detected in bursectomized agammaglobulinemic chickens, excluding the possibility that the receptors characterized in this study were synthesized by T cells.

Moreover, the metabolic turnover of membrane bound receptor was exactly the same as M-Ig (2.3:1.8) and much smaller than secreted antibodies. We assume that receptors and antibodies are synthesized by specific antigen binding cells, whereas Ig's are synthesized by other B cells. Nevertheless, there was no difference in the turnover between M-Ig and receptors, or between S-Ig and antibodies (Table II).

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References

Allan, D., and Crumpton, M. J. (1971), *Biochem. J. 123,* 967.

Andersson, J., Lafleur, L., and Melchers, F. (1974), *Eur. J. Immunol. 4,* 170.

Arias, I. M., Doyle, D., Schimke, R. T. (1969), *J. Biol. Chem. 244,* 3303.

Bock, K. W., Siekevitz, P., and Palade, G. E. (1971), *J. Biol. Chem. 246,* 188.

Choi, Y. S., and Good, R. A. (1972), *J. Exp. Med. 135,* 1133.

Choi, Y. S., and Good, R. A. (1973), *J. Immunol. 110,* 1485.

Choi, Y. S., Knopf, P. M., and Lennox, E. S. (1971a), *Biochemistry 10,* 659.

Choi, Y. S., Knopf, P. M., and Lennox, E. S. (1971b), *Biochemistry 10,* 668.

Clawson, C. C., Cooper, M. D., and Good, R. A. (1967), *Lab. Invest. 16,* 407.

Cuatrecasas, P., and Parikh, I. (1972), *Biochemistry 11,* 2291.

Davie, J. M., and Paul, W. E. (1971), *J. Exp. Med. 134,* 495.

Dehlinger, P. J., and Schimke, R. T. (1971), *J. Biol. Chem. 246,* 2574.

Evans, W. H. (1970), *Biochem. J. 116,* 833.

Fiske, C. H., and Subbarow, Y. (1925), *J. Biol. Chem. 66,* 375.

Greaves, M. F., and Hogg, N. M. (1971), *Prog. Immunol., Int. Congr. Immunol., 1st, 1971 1,* 111.

Heppel, L. A., and Hilmoe, R. J. (1951), *J. Biol. Chem. 188,* 665.

Jamieson, J. D., and Palade, G. E. (1967), *J. Cell. Biol. 39,* 589.

Kiehn, E. D., and Holland, J. J. (1970), *Biochemistry 9,* 1716.

Kiszkiss, D. F., Choi, Y. S., and Good, R. A. (1972), *J. Immunol. 109,* 1405.

Knopf, P. M. (1973), *Transplant. Rev. 14,* 10.

Lopes, J., Nachbar, M., Zucker-Franklin, D., and Silber, R. (1973), *Blood 41,* 131.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265.

Marchalonis, J. J., Cone, R. E., and Atwell, J. L. (1972), *J. Exp. Med. 135,* 956.

Munro, H. N., and Fleck, A. (1966), *Analyst (London) 91,* 78.

Nason, A., and Vasington, F. D. (1963), in Methods of Enzymology, Colowick, S. P., and Kaplan, N. O., Ed., New York, N.Y., Academic Press, VI 409.

Neville, D. M., and Glossmann, H. (1971), *J. Biol. Chem. 246,* 6335.

Nordin, A. A., Cosenza, H., and Sell, S. (1970), *J. Immunol. 105,* 154.

Omura, T., Siekevitz, P., and Palade, G. E. (1967), *J. Biol. Chem. 242,* 2389.

Palade, G. (1959), in Subcellular Particles, Hayashi, T., Ed., New York, N.Y., Ronald Press, 64.

Phillips, D. R., and Morrison M. (1970), *Biochem. Biophys. Res. Commun. 40,* 284.

Ragland, W. L., Pace, J. L., and Doak, R. L. (1973), *Biochem. Biophys. Res. Commun. 50,* 118.

Schmidt-Ullrich, R., Ferber, E., Knufermann, H., Fischer, H., and Wallach, D. F. H. (1974), in Protides of the Biological Fluids, Peeters, H., Ed., New York, N.Y., Pergamon Press, 73.

Shibko, S., Koivistoinen, P., Tratnyek, C. A., Newhall, A. R., and Friedman, L. (1967), *Anal. Biochem. 19,* 514.

Singer, S. J. (1974), *Adv. Immunol. 19,* 1.

Thorsby, E., and Bratlie, A. (1970), in Histocompatibility Testing 1970, Terasaki, P. I., Ed., Copenhagen, Munksgaard, 55.

Touster, O., Aronson, N. N., Dulaney, J. Y., and Henrickson, H. (1970), *J. Cell. Biol. 47,* 604.

Vitetta, E. S., Baur, S., and Uhr, J. W. (1971), *J. Exp. Med. 134,* 242.

Warner, N. L. (1974), *Adv. Immunol. 19,* 67.

Warren, L., Glick, M. C., and Nass, M. K. (1966), *J. Cell. Physiol. 68,* 269.

Wigzell, H. (1970), *Transplant. Rev. 5,* 76.

Zimmerman, B. (1974), *J. Immunol. 112,* 249.