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DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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* * * * *

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Studies in Immunology. Professors M. Reins, B. Zwilling

Studies in Avian Diseases. Professors G. Marsh, Y. M. Saif
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INTRODUCTION

The efficient production of domestic animals relies partly upon reducing disease loss due to mortality and morbidity. It has been estimated by the World Health Organization (Pritchard, 1966) that in developed countries in Europe, Oceania, and Northern America, 17.5 percent loss of animal products comprising 4.06 million metric tons of animal protein is lost annually because of disease problems. It was estimated that 35 percent of all animal products or approximately 6.71 million metric tons of animal protein is lost in the remaining world, consisting of most of the developing countries. In the areas of intensive poultry production most mortality and morbidity occur because of neonatal infection. Thus, there has been great interest among scientists in neonatal infection and immunity, resulting in the design of disease control programs for the specific problems found in young poultry.

The acquisition of immunity in any young animal consists of two distinctive overlapping phases, the acquiring of maternal immunity and the development of active immunity. The importance of these phenomena is dramatically illustrated when impairment of either of these protective mechanisms occurs. The result of such immunological deficiencies is often neonatal death due to overwhelming microbial infection. Examples of such deficiencies may involve the T and B
lymphocytes, the effector cells of the immune response, or deficiencies in acquiring maternal antibodies because of poor gut absorption of colostrum (Eisen, 1973; McGuire et al., 1974, 1977). Maternally acquired immunity (also called maternal or congenital passive immunity) is transient and involves the passage of specific antibodies or in some mammalian cases immune cells (lymphocytes and macrophages) produced in the dam and transferred by one of several routes to the embryo, fetus, or neonatal animal (Brambell, 1970; Parmely and Beer, 1977). In some species one or more Ig classes are favored in the transfer to the neonate. Irregardless of the specific method of transfer, the result is that the collective immunological experience of the dam is transferred to the offspring (Brambell, 1970).

The development of the active immunity in the neonatal animal is the result of the interaction of antigens in the environment and the developing immune "apparatus". The immunological "apparatus" consists of certain cells of the monocytic cell line, B lymphocytes, T lymphocytes, and macrophages. These cells act cooperatively to recognize foreign antigens, produce specific antibodies, mount "cellular" immune response involving the production of "killer" lymphocytes, cytotoxic substances, and the activation of accessory phagocytic cells (Eisen, 1973). The development or ontogeny of active immunity is not the subject of this monograph but interested readers may refer to several extensive reviews (Good and Papermaster, 1964; Greaves et al., 1974). It is of interest, however, to note that maternal passive antibody to specific antigens can interfere or suppress the active antibody responses to the corresponding antigens.
The presence of maternal immunity in birds and its protective and suppressive roles are well documented (Hofstad et al., 1972; Brandly et al., 1946; Hallauer, 1936; Rosenberger, 1975; Richey and Schmittle, 1962; Raggi and Lee, 1958, 1965) and are an important influence in epizootic and enzootic disease patterns, and in the consideration of the design of the disease control programs.

Brambell (1970) has reviewed maternal immunity in the fowl, and indicated that serum gammaglobulins are transferred across the hens follicular epithelial cells into developing ova. Later during embryonation, and for a short period after hatching, gammaglobulins cross the yolk sac endodermal epithelial cells into the embryonic or neonatal circulation by way of the vitellin circulation. Kramer and Cho (1970) have suggested another route of early maternal immunoglobulin G (IgG) absorption by the embryo. They have shown that IgG diffuses from the egg yolk to albumin, and into the amniotic cavity, where it gains access to the digestive tract of the embryo.

Immunoglobulin classes G, M, and A have been isolated in the chicken with IgG being the predominant Ig in the serum (Leslie, 1975). IgG is also the predominant Ig class transferred to egg ova; however, IgM and IgA are reportedly present in unincubated egg yolk at lower concentrations (Leslie, 1975; Yamamoto, 1975; Orlands and Rose, 1972). Unincubated egg white contains all three Ig classes at low concentrations (Yamamoto, 1975; Rose and Orlands, 1974).

It is important to obtain additional information in other domestic avian species on the mechanism, amount, and kinetics of Ig transferred into ova and eventually into young birds. This report deals with
aspects of the metabolism and kinetics of passive transfer of radioactive $^{125}$I labeled IgG, IgM, and IgA into eggs in the laying turkey hen. In addition, the question of how long maternal IgG exists in the young turkey poult and its contribution to the levels of IgG during early growth in this species will be examined.
The avian immunoglobulins

The isolation and characterization of the avian immunoglobulin proteins (Ig) was preceded by the earlier work on the human Ig's. The Ig's are synthesized and secreted by lymphocytes of the "Bone Marrow" line (termed B cells) that are found circulating or in fixed aggregations within tissues and organs. In mammals the lymph nodes, spleen, and bone marrow are major sites of Ig synthesis. These proteins have been divided into numerous classes based upon differences in chemical, antigenic, functional, and distributional criteria. Briefly five classes or isotypes of human Ig's have been described, IgG, IgM, IgA, IgD, and IgE. All isotypes have the same ability to specifically bind ligands (Eisen, 1973).

IgG is the major Ig found in serum and consists of a monomeric form with molecular weight of 150,000 daltons. The molecule consists of four large peptide subunits, consisting of two identical light chains each with a molecular weight of 25,000 daltons, and two identical heavy chains each with a molecular weight of 50,000 daltons. The subunits of the molecule are joined by interchain disulphide bridges through cysteine residues and by interchain nonconvalent hydrophobic bonds (Roitt, 1974). Enzymatic degradation studies using the proteolytic enzymes papain or pepsin have shown that one "end" or
piece of the IgG molecule consisted of the two light chains and segments of the two heavy chains. These were called the Fab or F(\(ab\))\(_2\) pieces ('Fragment antigen binding') and contain the ligand binding sites. Another fragment was generated by enzymatic degradation called the Fc ('Fragment crystalizable') piece which is responsible for varied biological and effector functions, i.e., membrane transport, complement fixation. It was further found that all Ig isotypes once obtained in monomeric form by mild reductions were similar in structure to the four chain IgG model (Eisen, 1973).

IgM is the largest Ig and is found in serum as a pentamer, with a total molecular weight of 900,000 daltons. IgA exists in multiple forms, 160,000-380,000 daltons and is the predominant Ig of exocrine secretions of the mucous membranes. IgD and IgE are formed in lower concentrations in human serum. IgE is involved with immediate hypersensitivity or Type I reactions existing as a monomer with molecular weight of 200,000 daltons. IgD at approximately 185,000 daltons seems to be associated with lymphocyte surface membranes during ontogeny of B cells (Roitt, 1974). Isotypic subdivisions of the major Ig classes termed subclasses exist and were identified by antigenic and functional differences associated with the Fc portion of the molecule. Four subclasses of IgG (\(\gamma_1 - \gamma_4\)) and 2 subclasses of IgA (\(\alpha_1, \alpha_2\)) have been found in man. Further antigenic subdivisions exist within the Ig subclasses, in individual species. These are called Ig allotypes and have been exploited by geneticists and immunologists in studies on the genetics and biosynthesis of Ig's. Within allotypes further antigenic subdivisions have been identified and are termed Ig
idiotypes. The idiotypic Ig antigenic differences are unique to individual clones of lymphocytes (Eisen, 1973; Roitt, 1974).

The isolation and characterization of avian Ig's have been the subject of extensive reviews (Higgins, 1975; Leslie, 1975; Kramer, 1973). Most of the work has been accomplished in the chicken since it has been used as a laboratory animal for many years. Some of the important characteristics of the avian Ig's are presented in Table 1.

IgG is the predominant class of serum and egg yolks in chickens with adult serum concentrations of 5.29±1.36 mg/ml reported (Leslie and Clem, 1970). It is also found in certain secretions such as seminal plasma, tracheobronchial washings, gastrointestinal tract secretions, crop washings (Leslie et al., 1971) and egg white (Yamamoto, 1975). IgG has been isolated and purified from chickens (Benedict, 1968), quail, pheasants (Leslie and Benedict, 1969), and turkeys (Saif and Dohms, 1976) using the combined methods of Sephadex G-200 gel filtration and DEAE ion exchange chromatography similar to the procedure originally employed by Benedict (1968) for purification of chicken Ig's. Duck IgG was purified by the combined methods of starch block electrophoresis and G-200 gel filtration (Grey, 1967a).

Although chicken IgG is analogous in function and structure (determined by enzyme degradation studies) to mammalian IgG, it possesses several unique physiochemical properties that have lead to the proposed designation of IgY by Leslie and Clem (1969). These properties include aggregation induced by high molar concentrations of salt, lack of significant immunological cross reactivity with mammalian IgG, higher carbohydrate concentration and molecular weight, lower
Table 1. Some reported physical and chemical characteristics of avian and human immunoglobulins.

<table>
<thead>
<tr>
<th>Species</th>
<th>IgG</th>
<th></th>
<th></th>
<th>IgM</th>
<th></th>
<th></th>
<th>IgA</th>
<th></th>
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<tr>
<td></td>
<td>mol. wt $X 10^5$</td>
<td>$S_{20}$ value</td>
<td>% total carbohydrate</td>
<td>mol. wt $X 10^5$</td>
<td>$S_{20}$ value</td>
<td>% total carbohydrate</td>
<td>mol. wt $X 10^5$</td>
<td>$S_{20}$ value</td>
</tr>
<tr>
<td>Chicken$^a$</td>
<td>1.6-1.7</td>
<td>6.9-7.7</td>
<td>1.4-6.0</td>
<td>6.3-9.5</td>
<td>16.7-19.5</td>
<td>2.6</td>
<td>3.5-3.6</td>
<td>11.9-16.2</td>
</tr>
<tr>
<td>Quail$^b,c$</td>
<td>1.7</td>
<td>7.1-7.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pheasant$^b,c$</td>
<td>1.7</td>
<td>7.1-7.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey$^d$</td>
<td></td>
<td>7.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16.1</td>
</tr>
<tr>
<td>Duck$^e$</td>
<td></td>
<td>7.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human$^f$</td>
<td>1.5</td>
<td>7.0</td>
<td>3.0</td>
<td>9.0</td>
<td>18.0</td>
<td>12.0</td>
<td>1.5-6.0</td>
<td>7.0-13.0</td>
</tr>
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isoelectric pH, more electro-negative charge, and different heavy chain peptide maps than mammalian IgG (Higgins, 1975).

Several reports have suggested that subclasses of IgG exist (Orlans and Rose, 1972; Wilkinson and French, 1968; Watanbe and Isayama, 1973), but Higgins (1975) has stated that the studies do not conclusively prove the existence of such IgG isotypes.

Investigations of the lower molecular weight gammaglobulins of ducks indicate that two distinct "IgG" isotypes exist, one with a 5.7 S sedimentation coefficient, the other with a 7.8 S value. The higher molecular weight form is structurally similar to mammalian and chicken IgG and constitutes most of the IgG in Muscovy ducks. However, equal concentrations of the isotypes are reported in Pekin and Mallard ducks (Grey, 1967a,b, 1969). Grey (1967) concluded from enzymatic degradation studies that the 5.7 S form had a shortened Fc piece compared to the 7.8 S form.

Like mammalian IgM, the avian macromolecule is the first produced in the primary immune response (Higgins, 1975). After exposure to antigen, IgM appears in the serum as early as the second day after antigen injection, peaking between 4 and 8 days after immunization (Benedict et al., 1963). Thereafter the levels of IgM antibody decrease and are replaced by antibody of the IgG class. Although its molecular weight and in some cases sedimentation coefficient is slightly lower than the mammalian IgM (Table 1) it cross reacts with antisera to mammalian IgM in the complement fixation test (Mehta et al., 1972).
IgM is found primarily in serum and in various external secretions at lower concentrations (Leslie et al., 1971). IgM is reportedly present in the egg yolk and white in low concentrations (Yamamoto, 1975). Because of the difficulty in obtaining large quantities of purified IgM few immunochemical studies have been conducted on this molecule (Higgins, 1975). However, one study involved the reduction of the intact molecule to monomeric units which yielded molecules of 175,000 daltons (Leslie and Martin, 1973).

Chicken IgA was first described in bile by Lebacq-Verheyden et al. (1972). Since that time numerous confirmations have been reported (Bienenstock et al., 1972, 1973; Orlans and Rose, 1972; Leslie and Martin, 1973). It is the predominant Ig in exocrine secretions of the chicken (Lebacq-Verheyden, 1972; Leslie and Martin, 1973). Like mammalian IgA there are varied polymeric forms found in the blood and secretions of chickens. In serum, the high molecular weight form is excluded with IgM on G-200 gel filtration while a low molecular weight form is found in the IgG containing peaks (Leslie and Martin, 1973; Higgins, 1976). Chicken biliary IgA, a high molecular weight form, was purified by G-200 and DEAE cellulose chromatography (Watanabe and Kobayashi, 1974). In addition to the high molecular biliary and serum forms, and the lower molecular weight serum form, an 11 S "secretory" form was found in the gut (Watanabe et al., 1975). Conflicting reports on the presence or absence of an avian analogue to mammalian secretory piece in biliary, serum, or intestinal IgA are found in the literature.
Turkey IgA exists in multiple forms in serum similar to the situation in chickens (Dohms et al., 1976). The biliary form was purified by G-200 and DEAE chromatography according to the methods of Watanabe et al. (1974). Using biliary IgA purified by G-200 gel filtration alone, Saif and Dohms (1976) found that the $S_{20}$ value was 16.1, similar to the reported value of chicken biliary IgA (Watanabe, 1974). Although two forms were found in the duodenum, a high molecular weight gut form was found to be chromatographically and antigenically indistinguishable from biliary and high molecular weight serum IgA as determined by immunodiffusion assay against rabbit anti biliary IgA (monospecific for the alpha chain). The low molecular weight duodenal form, probably a monomer, was found to be missing an antigenic component found in the biliary and serum IgA's. IgA has been identified but not purified in Japanese quail, pheasants, guinea fowl, and pigeons (Parry and Aitken, 1975).

**Passive immunity in birds**

The earliest demonstration of natural transfer of passive immunity in birds was accomplished by Klemperer in 1893. Hens actively immunized by tetanus toxin transferred antitoxic properties into eggs. He was the first to demonstrate that yolks and not the whites of eggs contained the antitoxin. Ramon (1928) confirmed Klemperer's initial findings and expanded observations indicating that the serum of chicks hatched from immune hens also had tetanus antitoxin and that titers were almost equal to yolk titers from the same hens. Both Klemperer
and Ramon referred to this phenomenon as "inheritance" of immunity or as "immunity inheritance". Using diphtheria immunized chickens, Dziergowski (1901) showed that antitoxin passed from the serum into the developing ova in the Graafian follicle. During incubation, antitoxin passed from the yolk into the embryo during the first ten days with antitoxin also diffusing into the albumin. Dziergowski (1901) emphasized that "immunity inheritance" could better be described as congenitally acquired passive immunity involving the transfer of globulins from immune hen's serum. Jukes and Kay (1932), and Jukes et al. (1934) using chickens, and Fraser et al. (1934) using ducks demonstrated that antitoxin was associated with the maternal serum globulin and the egg yolk livetin fraction. These workers observed that serum and yolk antitoxin levels from individual hens fluctuated together. Jukes et al. (1934) stated, "There was some indication of a parallel relationship between the concentration of antitoxin in the blood serum and its concentration in the livetin fraction of yolk."

The transient nature of naturally acquired congenital immunity was demonstrated by several workers using challenge studies of hatched congenitally immune chicks with diphtheria (Ozawa, 1936), fowl plague (Hallauer, 1936), and fowl spirochetosis (Levaditi, 1906; Kroo and Orbaneja, 1935). These workers showed that serum antibody titers and resistance declined and later disappeared during the first three to four weeks of life. It was apparent by the 1930's that antibodies of the immune hen were transferred functionally unaltered to the yolk, and into the developing embryo and hatched chick, providing passive protection to toxic, viral, and bacterial challenges.
Expansion of information on the transfer of antibodies to egg yolk or chick serum was provided by numerous workers. The detection of antibodies to Newcastle Disease Virus (Brandly et al., 1947), Rous Sarcoma Virus (Andrews, 1939) and Infectious Bronchitis Virus (Jungherr and Terrell, 1948) were observed in chickens. Antibodies to Western Equine and St. Louis Encephalitis Viruses were observed in the egg yolks of such diverse avian species as the Western Mourning Dove, the Common House Finch (Reeves et al., 1954) the pigeon (Sooter et al., 1954 and the White Ibis (Kissling, 1954).

Research into passive immunity of the avian species after the 1930's utilized more sophisticated techniques and essentially confirmed previous work. In addition, the role of passively acquired immunity in specific disease problems of young poultry was studied. These studies fell into three general categories: 1) the resistance to infection after challenge with pathogens in chicks from immune hens, 2) the role of passive antibody in suppression of active antibody responses to specific virus pathogens, and 3) the identification of the antibody classes and the time sequences of antibody passage from hen to ova, embryo, and chick.

The protective effects of maternal Ig have been best demonstrated using certain viral pathogens. In general, partial or complete protection from disease and mortality was achieved from one to five weeks after hatching in congenitally immune chicks; however, numerous variables must be considered in the evaluation of the efficacy of maternal immunity. The immune status of hens must be evaluated for the level (titer) and type of antibody present. Richey and Schmittle
(1962) used chicks with different degrees of immunity derived from different hens and found differing responses to challenge and vaccination. The time of challenge is a critical factor since maternal antibody is metabolized over the first few weeks of life. Patterson et al. (1962a) found that gammaglobulin in hatched chicks had a three day half life. Thus, substantial differences in levels of maternal Ig would be found as the chicks aged. The virulence, amount, and route of injection of the challenge virus are additional variables noticed in the following studies.

Partial or complete protection from mortality was observed in congenitally immune chickens to the following viral pathogens:
Newcastle Disease Virus (Lancaster, 1964), Infectious Bronchitis Virus (Hofstad, 1972), Avian Encephalomyelitis Virus (Calnek et al., 1961), Fowl Plague Virus (Haller, 1936), Infectious Bursal Disease Agent (Hitchner, 1972; Rosenberger, 1975), Avian Lymphoid Leukosis Virus (Burmester, 1955; Burmester et al., 1956) and Merek's Disease Virus (Spencer and Robertson, 1972). Similar findings were observed in congenitally immune ducklings challenged with Duck Virus Hepatitis (Levine, 1972) and Duck Plague Virus (Leibovitz, 1972).

Resistance to challenge with bacterial and mycoplasmal pathogens in congenitally immune chicks is less well studied. As stated above Levalette (1906) and Kroo and Orbaneja (1935) found protection to challenge with *Borrelia* in maternally immune chicks. Buxton (1952) found antibodies to *Salmonella pullorum*, *S. typhi*, and *S. gallinarum* were passed from hens to eggs and subsequently to chicks. However, Solomon (1971) has questioned the protective nature of antibody with
these infections. Mohamed and Bohl (1968) studying *Mycoplasma meleagridis* infections of turkeys concluded that maternal agglutinins, "apparently did not prevent the growth of mycoplasma or the development of airsac lesions". However, McCapes et al. (1967) demonstrated protection to paratyphoid challenge in turkey pouls hatched from hens immunized with the same paratyphoid serotype. It must be emphasized with all these studies that a particular type of antibody at a particular level may be required for protection. Lack of protection in the presence of positive serology may not warrant the exclusion of a protective role for maternal antibody. For example, the significance of relative affinity of antibodies in diseases has not been thoroughly examined. It was suggested in a study by Ahlshedt et al. (1974) that relative antibody affinity is involved in the protective capacity of antibody against bacterial infection.

The role of passive antibody in the suppression of active antibody responses to particular antigens has been known for some time. Hallauer (1936) found that the active antibody response to Fowl Plague was suppressed in chicks hatched from immune hens. This observation was confirmed by Brandly et al. (1946) using Newcastle Disease Virus. These authors observed increasing mortality after intravenous challenge as the initial vaccination time was moved closer to hatching. The obvious practical concern was that the suppression of active immunity after vaccination of immune chicks would lead to incomplete protection within flocks raised where Newcastle Disease was enzootic. These observed suppressions of antibody responses are well documented for Newcastle Disease Virus. Lancaster (1964) has reviewed the subject.
and reported that passive immunity reduced serologic responses to live lentogenic virus vaccination administered intravenously or subcutaneously. Studies by Richey and Schmittle (1967) with Newcastle Disease Virus and by Raggi and Lee (1958, 1965) with Infectious Bronchitis Virus showed that serologic responses to vaccination were inversely related to the quantity of maternal antibody in chicks. However, Raggi and Lee (1965) could not find any correlation between infectivity and serologic responses, between infectivity and challenge results, or between serologic responses and challenge results. The lack of correlation between serology and challenge results with Newcastle disease has also been discussed by Beard and Easterday (1967). This complicated phenomena may be clarified by recognizing that like mammals, birds have a functionally independent local immune system associated with the mucous membranes. Evidence for this was provided by the studies of Parry and Aitken (1973). Chemically bursectomized SPF chicks (antibody free) were immunized intraocularly with Newcastle Disease Virus. They showed that protection of these birds from intranasal challenge with velogenic Newcastle Disease Virus was as effective as normal SPF contemporaries immunized and challenged in an identical manner. They concluded that the resistance to challenge in the bursectomized group was due to local immunity in the respiratory tract. Additional evidence by Beard and Easterday (1967) using aerosol vaccination with Newcastle Disease Virus showed protection of birds against challenge with a virulent virus by the respiratory but not the intramuscular route. More recently Malkinson and Small (1977) have shown that passively injected antibody to
Newcastle Disease Virus protected against mortality but not infection in chicks challenged either in the upper or the lower respiratory tract. This agrees with previous work by Gagliardi and Irsara (1958) who showed replication of Newcastle Disease Vaccine Virus only in the upper respiratory tract of immune chickens challenged at that site. Beard and Brugh (1975) stated that passive immunity only limits viral dissemination but not infectivity after virus challenge. However, Malkinson and Small (1977) demonstrated that passive antibody suppressed active antibody response only when the virus was introduced in the lower respiratory tract (airsac inoculation) and not after virus was introduced in the upper respiratory tract (eye inoculation). They suggested that the difference in response was due to the greater normal transudation of passive HI antibody into the airsacs than into the more anatomically complicated upper respiratory tract where substantial local immune tissues are found (Hardarian gland and Bronchial associated lymphoid tissue). This finding is similar to a report by Kaltreider and Chan (1976) who found that dogs had an increasing IgG/IgA ratio on the secretions of mucous membranes as sampling moved from the upper to the lower respiratory tract. The lower respiratory tract was like serum in Ig composition. Thus, congenitally derived antibody suppression seems to occur if antigen is injected into anatomical sites accessible to serum antibody. The above studies help explain the work of White et al. (1953) and Allan (1973) who showed that maternal antibody did not interfere with the acquisition of active immunity to Newcastle Disease when vaccination was by the intranasal, conjunctival, aerosol, or intraocular routes.
The characterization of antibodies transferred from the hen serum into egg yolk was hampered by the technical problem of antibody recovery from the complex yolk mixture. Egg yolks consist of approximately 50% solids. Most of the yolk solid constituents are lipoproteins of either high or low density. The high density lipoprotein is found in the form of granules comprising about 23% of the total yolk solids. The low density fraction consists of about 65 percent of the yolk solids. Recovery of the aqueous livetin or water soluble fraction containing antibody has involved either extraction with organic solvents (Kramer and Cho, 1970; Heller, 1975; Patterson et al., 1962a,b), precipitation lecitho-vitellin followed by recovery of livetin (Jukes et al., 1934) or by pelleting the granules and removing livetins by half saturated ammonium sulfate precipitation (Rose and Orleans, 1974). Three major livetin proteins were designated α-livetin, β-livetin, and γ-livetin because of their electrophoretic mobilities. These proteins have been shown by sedimentation, electrophoretic, immunologic and chemical methods to be identical with certain major serum proteins. Thus, α-livetin was associated with serum albumin, β-livetin is beta serum glycoprotein, and γ-livetin is gammaglobulin (Jukes and Kay, 1932; Williams, 1962; Nace, 1957; MacKenzie and Martin, 1967). Final proof of the serum origin of two of these proteins was provided by Patterson et al. (1962a,b) who showed that I\(^{131}\) labeled serum albumin and gammaglobulins injected into hens accumulated in yolks after transfer across the follicular epithelial cells.

Malkinson (1965) examined the antibodies to Escherichia coli by direct and indirect agglutination of alkaline extracted E. coli coated
erythrocytes. Although immune hen's serum produced "complete"
agglutinating antibody that was susceptible to 2-mercaptoethanol (2-Me)
dissociation (presumably macroglobulin, IgM), no 2-Me sensitive
antibody was found in yolks. In addition, this antibody was
"incomplete" in serologic testing and was suggested to be 7S
γ₂-globulin (IgG). Other support for the selective transfer of 7S
γ₂-globulin (IgG) into yolks was supplied by Orleans (1967) and Heller
(1975). However, Karthigasu et al. (1964) reported the detection of
19S 2-Me sensitive (presumably IgM) and 7S 2Me resistant (presumably
IgG) opsonins to gram negative bacteria in the serum of embryos while
only the 7S antibody was found in newly hatched chicks. None of the
above studies conclusively demonstrated the identity of the passively
transferred Ig classes since monospecific antisera to Ig isotypes were
not available. Kramer and Cho (1970) used antisera monospecific for
IgG and IgM to identify egg yolk and white Ig's. Only IgG was found
in egg yolk. This was confirmed by Faith and Clem (1973), and by
Rose et al. (1974). Yamamoto et al. (1975) identified all three Ig
classes in egg yolk and white but detection of IgM and IgA required
tenfold concentration of yolk material. It would appear that yolk IgG
is by far the predominant and most important Ig class in the yolk.

The time of the passage of Ig into the hen's egg has been revealed
by the isotope tracer studies of Patterson et al. (1962a,b).
Radioactivity was observed to accumulate in egg yolks after
intravenous injection of 131I labeled gammaglobulin into laying hens.
The first egg layed after injection of the isotope had no radioactivity
since it was in the oviduct at the time of injection. Thereafter,
increasing amounts of radioactivity associated with gammaglobulin were observed in the yolks of sequentially laid eggs, peaking at 5 days after injection. This observation agrees with reports that antibody titers in egg yolks peaked 5 days after peak titers were observed in serum (Patterson 1962b; Orlans, 1967). By using a single injection of labeled gammaglobulin, followed by the sacrifice of the hens at 24 hours later, Patterson et al. (1962a,b) were able to determine the transfer rate per cm² of yolk surface for individual ova at different stages of growth in the ovary. They found an increase in transfer rate as ova began the rapid development phase of growth and a decline in the transfer rate of the largest 2-3 ova found in the ovary. Maximum passage of labeled gammaglobulin occurred in the middle size ova (3-8 ml volumes) corresponding to ova within 4 days of ovulation.

Morphological changes were also observed in the follicular epithelial cells during the time of maximum transfer. It was apparent from these experiments that the 5-day delay of peak levels of radioactivity in sequentially laid eggs was due to the fact that the middle size ova, having the highest rate of gammaglobulin transfer, were exposed to the highest serum levels of label for the longest period of time. Thus, the time of delay before peak yolk antibody levels after peak serum levels occur is a function of the number of eggs in rapid development.

It is also apparent that the transfer of gammaglobulin in the chicken is similar to the transport of the other macromolecular constituents of the egg yolk and with the total ova growth during the final phase of rapid development (MacKenzie and Martin, 1967; Warren and Conrad, 1939).
The persistence of antibody in egg yolk under storage conditions or during embryonation appears to be unaffected by proteolytic enzymes known to be present in egg yolk (Kramer and Cho, 1970). Brandly et al. (1946) found that no loss of antibody titer to Newcastle Disease Virus occurred after storage at 4-6 C for as long as six months. During embryonation Brierly and Hemmings (1956) found that levels of agglutinins to bacteria were not lowered. Buxton (1954) stated that, "practically no alteration in the quantity or type of antibody transferred from the yolk to the embryo" occurred after studying the passage of agglutinating and nonagglutinating antibodies to Salmonella. Kramer and Cho (1970) have suggested that IgG may be partially degraded by proteases since they found shortened \( \gamma_2 \) precipitin band in embryo serum and albumin during embryonation. However, quantitative estimation of protein degradation was not attempted in this study. Finally, Brierly and Hemmings (1956) incubated immune serum with sterile unincubated and six day incubated egg yolk at 39 C for 13 days and found no loss in antibody titer.

The time and route of transfer of Ig and antibodies from the yolk was the subject of an earlier review by Brambell (1970). The earliest reported appearance of serum antibody to Salmonella was 11 days after the beginning of incubation. Titers were also found from 11 through to the 17th day of incubation (Buxton, 1954). However, a Newcastle Disease Virus neutralizing substance from embryonic tissues and extraembryonic membranes has been found from 6-9 days of incubation by Brandly et al. (1946). These workers did not detect antibody to virus in embryonic serum until the 18th day of incubation. Kaminski and
Durieux (1956) found gammaglobulin in embryonic serum on the 14th day of incubation while Nace (1953) detected gammaglobulin by immune precipitation and Kramer and Cho (1970) detected IgG by immunoelectrophoresis on the 12th day of incubation. Leslie and Martin (1973) quantitated levels of IgG at 15 days incubation and found levels to be 2-4% of the hatching serum IgG concentration. This data agrees with the previous work of Marshall and Deutch (1950) who found a 3-4 fold increase in gammaglobulin in serum in the few days prior to hatching. Brierly and Hemmings (1956) have shown that antibody is transferred from the retracted yolk sac after hatching at a rate equal to that found at the end of incubation. The duration of this transfer after hatching is dependent upon the rate of absorption of the yolk sac. From these studies in chickens it would appear that antibody is passed from egg yolk to embryonic serum around day eleven. The amount of antibody increases until sometime around hatching; thereafter transfer continues from the retracted yolk sac for a short time after hatching.

The literature on the relation between serum and egg yolk levels of antibody from individual hens is confusing. Brambell has cited research workers who have shown that egg yolk titers were found to be from 13-100 percent of the serum antibody titers of corresponding hens. In part, this may be due to the variability in the percent recovery of antibody from the yolk material, a variable that was often not evaluated. It is also recognized that there are varying sensitivities in the serological assays employed in these studies, i.e., virus neutralization versus bacterial agglutination. Another possible source of this variability was suggested by Heller (1975). He has reported
that individual hens transferred variable amounts of antibody into sequentially laid eggs. Differences in the levels of transfer of antibody between different hens has been reported but not thoroughly investigated (Kramer and Cho, 1970).

The pathway or route of transfer of Ig from the egg yolk into the embryo is believed primarily to occur via the yolk sac endodermal epithelial cells and the vitelline circulation. Although the yolk sac is attached to the intestine by the yolk sac stalk, contradicting reports exist on the transfer of yolk substances directly into the gut. Romanoff (1960) discussed instances where yolk was found in the lumen of the yolk sac stalk and in the embryonic intestine, while Lillie (1927) contradicts this assertion. Without evidence, Karthigasu et al. (1964) stated that IgM is passed from the yolk sac directly into the embryonic gut. Kramer and Cho (1970) have shown the presence of IgG antibody in the intestine of the chicken embryo during incubation; however, the origin of this Ig could not be determined. Finally, two authors failed to detect serum titers after oral administration of immune serum into hatched chicks (Buxton, 1952; Brierly and Hemmings, 1956). Although Kramer and Cho (1970) have shown a "circuitoous" route of embryonic IgG absorption by diffusion from the ruptured vitelline membrane through the extraembryonic fluids, it would appear from an analysis of the above reports that most absorption occurs by way of the yolk sac vitelline circulation.

The metabolism of immunoglobulins

Part of the biological behavior of plasma proteins includes their metabolism and distribution within the vertebrate organism. Serum or
plasma levels of a particular protein are dependent upon the rates of synthesis and catabolism of the protein. The metabolism of Ig's has been studied in great detail in humans because of the interest in certain metabolic disorders associated with these proteins (Waldman et al., 1971). Studies of the metabolism of the Ig's in a wide variety of animal species have also been conducted (Table 2).

Generally, five methods of studying Ig metabolism have been employed by workers using neonatal and adult animals. The methods include: (1) monitoring the elimination of passively injected antibody, (2) quantitating the loss of maternally acquired Ig in neonatal animals, (3) injection of homologous radioactively labeled Ig preparations and subsequent study of the elimination of radioactivity from blood, urine, or the whole body, (4) pulse labeling of an animal with a radioactive protein precursor, i.e., a C14 amino acid, followed by monitoring the decline of newly synthesized internally labeled Ig, and (5) the use of constant infusion of known levels of radioactive labeled Ig to evaluate fluxuations in the total pool turnover rate.

The first four experimental methods of study rely upon an observed linear exponential loss of plasma activity over a time period that follows the kinetics of first order reactions (Lehninger, 1972). This form of kinetics can be expressed as

\[ A \rightarrow P \]  

where the reaction rate is proportional to the concentration of either A (initial Ig level) or the product, P. In the case of Ig catabolism, no final product is formed, since Ig is believed to be catabolized to the level of amino acids. Thus, the reaction is proportional to the
Table 2. Serum concentration, half-life, fractional turnover, and total turnover rate of antibodies, gammaglobulins, or immunoglobulins in various animal species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Protein</th>
<th>Serum conc. mg/ml</th>
<th>$T_\frac{1}{2}$ days</th>
<th>Fractional turnover rate</th>
<th>Total turnover rate mg/kg/day</th>
<th>Reference</th>
</tr>
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<tr>
<td>Human</td>
<td>$\gamma$-globulin</td>
<td>20.3</td>
<td></td>
<td></td>
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<td>Dixon et al. (1952)</td>
</tr>
<tr>
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<td>$\gamma$-globulin</td>
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<tr>
<td></td>
<td>IgG</td>
<td>12.1</td>
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<td>0.370</td>
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<td>0.720</td>
<td>0.002</td>
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<td>Serum IgG_2</td>
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<td>0.088</td>
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<td>Cripps and Lascelles (1974)</td>
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</table>
Table 2. (con't) Serum concentration, half-life, fractional turnover, and total turnover rate of antibodies, gammaglobulins, or immunoglobulins in various animal species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Protein</th>
<th>Serum conc.</th>
<th>(T^\frac{1}{2}) days</th>
<th>Fractional turnover rate</th>
<th>Total turnover rate mg/kg/day</th>
<th>Reference</th>
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<td>Monkey</td>
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<td>6.6</td>
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<td>5.6 ±.12</td>
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<td>.57-.61</td>
<td>3.0 ±0.1</td>
<td>.40±.02</td>
<td>.40±.02</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>IgG myolomas:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fahey and Sell (1965)</td>
</tr>
<tr>
<td></td>
<td>7S $\gamma_{2a}$</td>
<td>4.0</td>
<td>4.8-5.4</td>
<td>.13</td>
<td>______</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7S $\gamma_{2b}$</td>
<td>4.0</td>
<td>2.5-3.0</td>
<td>.25</td>
<td>______</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7S $\gamma_{1}$</td>
<td>2.5</td>
<td>4.0</td>
<td>.17</td>
<td>______</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgA myolomas:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fahey and Sell (1965)</td>
</tr>
<tr>
<td></td>
<td>$\gamma_{1a}$</td>
<td>0.4</td>
<td>1.0-1.3</td>
<td>______</td>
<td>______</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\beta_{2}$</td>
<td>1.3</td>
<td></td>
<td>______</td>
<td>______</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgM ($\gamma_{1M}$)</td>
<td>_______</td>
<td>0.5</td>
<td>______</td>
<td>______</td>
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</tr>
</tbody>
</table>
Table 2. (con't) Serum concentration, half-life, fractional turnover, and total turnover rate of antibodies, gammaglobulins, or immunoglobulins in various animal species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Protein</th>
<th>Serum conc. mg/ml</th>
<th>T½ days</th>
<th>Fractional turnover rate</th>
<th>Total turnover rate mg/kg/day</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat, adult</td>
<td>γ-globulin</td>
<td>_____</td>
<td>5.5</td>
<td>_____</td>
<td>_____</td>
<td>Bangham and Terry (1957)</td>
</tr>
<tr>
<td>Rat, neonate</td>
<td>γ-globulin</td>
<td>_____</td>
<td>5.5</td>
<td>_____</td>
<td>_____</td>
<td>Humphrey (1961)</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>γ-globulin</td>
<td>_____</td>
<td>12.0</td>
<td>_____</td>
<td>_____</td>
<td>Dixon et al. (1952)</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>γ-globulin</td>
<td>_____</td>
<td>5.4</td>
<td>_____</td>
<td>_____</td>
<td>Dixon et al. (1952)</td>
</tr>
<tr>
<td>Guinea pig, noenate</td>
<td>γ-globulin</td>
<td>_____</td>
<td>7.0</td>
<td>_____</td>
<td>_____</td>
<td></td>
</tr>
<tr>
<td>Chicken, adults</td>
<td>γ-globulin</td>
<td>_____</td>
<td>4.3 ±.15</td>
<td>_____</td>
<td>_____</td>
<td>Westman and Olson (1964)</td>
</tr>
<tr>
<td>Chicken, 4-week</td>
<td>γ-globulin</td>
<td>_____</td>
<td>2.4 ±2.9</td>
<td>_____</td>
<td>_____</td>
<td>Phillips (1966)</td>
</tr>
<tr>
<td>Chicken, hen</td>
<td>γ-globulin</td>
<td>_____</td>
<td>1.46±.3</td>
<td>_____</td>
<td>_____</td>
<td>Patterson et al. (1962a, b)</td>
</tr>
<tr>
<td>Chick, neonate</td>
<td>γ-globulin</td>
<td>_____</td>
<td>3.0</td>
<td>_____</td>
<td>_____</td>
<td>Patterson et al. (1962a)</td>
</tr>
<tr>
<td>Chicken, male</td>
<td>IgG</td>
<td>5.0</td>
<td>4.1</td>
<td>.169</td>
<td>58.0</td>
<td>Leslie and Clem (1970)</td>
</tr>
<tr>
<td>Chicken, male</td>
<td>IgM</td>
<td>_____</td>
<td>1.7</td>
<td>.41</td>
<td>16.0</td>
<td></td>
</tr>
</tbody>
</table>
rate of disappearance of A, the Ig concentration, at any given time. This is expressed as

\[-\frac{d[A]}{dt} = k[A]\]  \hspace{1cm} (2)

where [A] is the concentration of Ig and \(-d[A]/dt\) is the rate at which [A] decreases. The proportionality constant \(k\) has dimensions in reciprocal time such as \(sec^{-1}\), \(min^{-1}\), or \(days^{-1}\). The slope (b) of the linear Ig specific radioactivity time curve is \(-d[A]/dt\). The slope (b) can be determined experimentally by plotting the plasma activity loss over the experimental period. Using mathematical integration, equation (2) can be written as

\[
\log_{10} \frac{[A_0]}{[A]} = k t \hspace{1cm} (3a)
\]

where \([A_0]\) is the concentration of A at time 0 and [A] is the concentration at any time, \(t\). It can also be written as

\[
b = \frac{kt}{2.302} \hspace{1cm} (3b)
\]

Thus, at any time,

\[
k = b \times 2.302 \hspace{1cm} (3c)
\]

Physiologists and biochemists have designated the proportionality constant for first order reactions, \(k\), as the fractional turnover rate, since it expresses the rate of loss of Ig in time. This term multiplied by 100 provides a convenient way of expressing fractional loss as the percent loss of Ig from the plasma pool per unit time.

Absolute turnover rate in moles or grams lost per unit body weight per unit time is determined by the following equation:
Total Turnover Rate = \( k \times M \) \hspace{1cm} (4)

where \( k \) is the fractional turnover rate and \( M \) is the total plasma pool concentration of Ig. In single injection experiments, equilibration of the injected material throughout the body occurs before linear exponential loss is observed. During the linear phase of elimination it is assumed that equilibration between all body pools is at a constant rate. Therefore, equation 4 reflects the total body turnover rate of Ig. If the animal is in steady state with regard to Ig metabolism, then the total turnover rate is also the total synthetic rate of Ig, excluding the synthesis of Ig from submucosal tissues delivered directly into external secretions (Waldmann et al., 1971).

Many researchers have utilized a more convenient method of expressing Ig catabolism, the half-life or half-time. The half-life is the time necessary for one-half of the original Ig concentration to be eliminated by catabolism and is expressed by the following equation:

\[
t^{1/2} = \frac{0.693}{k}
\]  \hspace{1cm} (5)

where \( t^{1/2} \) is the half-life and \( k \) is the fractional turnover rate.

Detailed mathematical treatment of the above discussion can be found in the review of Shipley and Clark (1972).

Early work on Ig metabolism utilized the method of injecting specific antibody into man or animals to study the catabolism of the particular antibody (Perkins et al., 1958; Wiener, 1951; Barr et al., 1949; Dixon et al., 1952; Kerr and Robertson, 1954). The principle advantage of this type of study was that little or no potentially
denaturing chemical manipulations were required in preparing the injected antibody. The disadvantage of this method involved the sensitivity of the serological technique employed in detecting the decline of plasma activity. Often, however, virus neutralization (Perkins et al., 1958) or antitoxin assays (Barr et al., 1949), among the more sensitive serological tests, were employed.

The persistence of maternal antibody is dependent not only on the amount of Ig passed to the neonate but upon the endogenous catabolic rate of the Ig in the neonate. When injecting neonatal animals with antibody, or in catabolic studies of congenitally acquired antibody, problems can be encountered when consideration is not taken for increasing blood volume during growth (Solomon, 1971). This increase in blood volume is particularly evident in domestic birds that double in body weight and blood volume every seven days (Medway and Kane, 1954). Thus, growth dilutes plasma specific activity of injected antibody, radioactively labeled Ig, or maternally derived antibody. A similar dilutional problem may be encountered using a quantitative immune assay to evaluate the decline in maternally acquired Ig.

Another problem encountered with the quantitative immune assay method in neonatal animals involves the onset of in situ Ig synthesis. The problem only exists if neonatal synthesis of a particular class of Ig cannot be distinguished from the maternal isotype. The studies of Curtis and Bourne (1971, 1973) illustrate this problem. Using radial immune diffusion assay for studying the decline of IgG, IgM, and IgA in colostrum fed neonatal pigs, these workers recorded half-lives of 12-14, 4.5 and 3.5 days respectively (Curtis and Bovine, 1971). In a
later paper (Curtis and Bourne, 1973), half-lives were determined using
the method of single injection of $^{125}\text{I}$ labeled IgG, IgM, and IgA and
compared to the previous method. The IgG and IgA half-lives were the
same for both techniques; however, the IgM half-life determined by
immune assay was longer than the half-life result of the isotope
method. This result was interpreted to mean that IgM synthesis had
begun in the neonate during the experiment. Thus, the quantitative
immune assay technique utilized alone in rapidly growing species may
lead to erroneously prolonged half-lives of Ig's.

Single injection isotope experiments have been the method of
choice for determining half-life of Ig classes. All data in Table 2
not designated in the second column as "antibody" utilized the single
injection of labeled gammaglobulin or purified immunoglobulin isotypes
in metabolic studies. Most researchers have labeled Ig using a
modification of the external radiiodination technique of Hunter and
Greenwood (1962). The use of Ig labeled with $^{125}\text{I}$ or $^{131}\text{I}$ gamma
emitters has the advantage of obtaining reagents with high specific
activity that show limited reincorporation into other proteins after
in vivo catabolism. Nonradioactive dietary iodine provided in excess
allows the excretion of metabolically liberated free radioactive iodine
without the reincorporation into the thyroid hormones (Cohen et al., 1956).

The original method of iodine labeling involved the use of a
strong oxidizing agent such as chloramine T to bond the label to
tyrosine residues of the Ig. Some workers have suggested and
demonstrated that this oxidation can cause denaturation or alteration
in the metabolic behavior of the labeled product (Berson et al., 1953; Marchalonis, 1969). However, the metabolic studies by Freeman (1959) and Cohen et al. (1956) compared oxidatively iodinationed albumin or gammaglobulins with C\textsuperscript{14} internally labeled molecules. Cohen et al. (1956) showed that no difference was observed between C\textsuperscript{14} labeled molecules and iodine labeled molecules during metabolism. Freeman (1959) showed that accelerated catabolism only occurred after 2.9 or greater atoms of iodine were present per albumin molecule. Thus, over iodination can substantially effect the metabolic behavior of a protein. Recently the enzymatic lactoperoxidase technique of iodinating Ig described by Marchalonis (1969) has been widely used. The principle advantage is that the iodination reaction takes place at physiological pH without the use of a strong oxidizing agent. However, Levy and Davison (1976) have shown that both the chloramine T and lactoperoxidase labeling techniques interfered with antibody binding activity of IgG. The exact reason or significance of this phenomena for metabolic studies is not known. Wostman and Olson (1964) utilized internally labeled Ig, prepared by C\textsuperscript{14} glycine pulse labeling, to follow the elimination of newly synthesized antibody. This technique necessitated the reisolation of gammaglobulin before antibody determination since C\textsuperscript{14} glycine was incorporated into a wide variety of serum proteins. This technique provided the advantage of internal labeling but the reisolation technique was not evaluated for recovery and purity of gammaglobulin.

Several assumptions must be made in a single injection experiment. These are that exchange rates of Ig between body pools, pool size of
Ig (rate of synthesis), and body compartment size remain constant during the experiment. These cannot be directly examined by this method. Cohen (1956) studying the plasma protein distribution in the follicular and luteal phases of the female baboon menstrual cycle, and Sasaki (1976) studying prepartum and postpartum cows, both found that new body compartments were opened during the experiments. Swelling of the baboon perineal sex tissue allowed considerable redistribution of plasma proteins; similarly, in the cow large amounts of IgG entered the mammary gland from the serum prior to parturition. These parameters can be examined directly by using a constant infusion of isotope followed by analysis of the activity of labeled Ig per unit Ig in the sample. The changes in pool size, synthetic rates or exchange rates between pools were directly examined in dairy cows around parturition using this method (Sasaki et al., 1976).

It is apparent from the data in Table 2 that within a particular species, half life differences are observed between Ig classes. IgG in all species examined had the longest half-life and lowest turnover rate while IgM, IgA, IgD, and IgE had comparatively shorter half-lives and greater turnover rates. Within subclasses of human, bovine, ovine and caprine IgG there are observed differences in catabolic rates. Morrell et al. (1970) found that human IgG₁, IgG₂, and IgG₄ had similar fractional turnover rates of 7 percent of the total intravascular pool per day, while IgG₃ had a 17 percent fractional turnover. Similar relationships were found in IgG₁ and IgG₂ of ungulates (Table 2).

Human IgG, IgA, and IgE are generally distributed equally between the intravascular and extravascular spaces. However, IgM and IgD are
distributed mostly in the intravascular space with 76 percent of the IgM and 75 percent of the IgD normally found within the vascular system (Waldman et al., 1971). A similar distribution for IgM has been reported in rabbits with 71 percent of the IgM found distributed intravascularly (Sabaston and Ste Rose, 1976). The reason for this is not known but may reflect differences in molecular weights of these Ig's (Table 1).

The catabolism of IgG appears to be associated with the Fc portion of the molecule. Spiegelberg and Weigle (1965) and Wochner et al. (1967) have both shown that the metabolism of the Fc portion of the molecule, obtained by papain or pepsin, is similar to the metabolism of the intact IgG molecule. The metabolism of light chains or the Fab fragments have considerably shorter half-lives than the intact IgG. Furthermore, the Fc piece is transported across the intestinal membranes of the neonatal rat while the Fab piece is not transferred (Waldman et al., 1971).

Excluding the changes in pool size, several physiological factors can affect the metabolism of Ig's. These include, (1) synthetic rates of Ig and therefore the plasma pool concentration of Ig, and (2) basal metabolic rate. Waldman et al. (1971) has discussed the 500 fold range in the rate of synthesis of different Ig classes in germfree mice versus hyperimmunized conventional mice. Rate of synthesis may effect the catabolic rate since it has been shown that the rate of IgG catabolism is directly related to IgG concentration (Brambell, 1964, 1970; Fahey and Robinson, 1963; Fahey and Sell, 1965). However the catabolic rates of IgM and IgA are independent of their Ig
concentration (Waldman et al., 1971). Thus, the survival of IgG is prolonged at low serum concentrations and shortened at higher concentrations. Brambell (1964, 1970) has developed a mathematical model for the "concentration-catabolism" effect of IgG and has presented an explanation of the phenomena. He suggests that at all times a fraction of the IgG plasma pool is isolated into a catabolic pool (presumably intracellular vesicles). Within the metabolic pool certain IgG receptors bind some portion of the isolated IgG fraction and are later returned to circulation. The IgG fraction not bound to receptors is degraded by enzymes. At high IgG concentrations the receptors become totally saturated resulting in the catabolism of the remaining large proportion of the isolated pool. The "concentration-catabolism" phenomena for IgG has been observed in mice and rabbits (Fahey and Sell, 1965; Sabiston and Ste. Rose, 1976).

Hypermetabolism associated with fever, or injection of thyroxin, or the injection of corticosteroids have all been shown to increase Ig turnover (Waldman et al., 1971; Levy and Waldman, 1970). Sabastion and Ste. Rose (1976) have shown that prolonged cold exposure of rabbits had an accelerating effect on turnover rates for both IgG and IgM when compared to non-stressed controls. Serum IgG and IgM concentrations were not different between stressed and non-stressed groups. This suggested that an increased rate of IgG and IgM synthesis occurred during the prolonged exposure to cold.

Two overall observations can be made from the different animal species used in experiments on metabolic rates of Ig's (Table 2). First, with decreasing species size it appears that Ig half-life and
turnover rates increase, presumably because of the increased basal metabolic rates found in smaller animals (Solomon, 1971). Secondly, half-lives of adult and neonatal animals of the same species are about the same. However, more extensive study using purified Ig classes and calculations of fractional and total turnover rates will be required to clarify the latter phenomena.
MATERIALS AND METHODS

Birds

Adult turkey hens were obtained from a strain of broad-breasted white turkeys genetically selected over 16 generations for increased egg production over commercial contemporaries (Nestor et al., 1970; Nestor, unpublished data). Egg production data indicate that this strain averaged 119 eggs/180 days, much higher than the 81 eggs/180 days of a commercial type randombred control. Although significant differences in body weight, egg weight, ovum weight, and number of eggs in rapid development were observed compared to a meat type turkey hen, no significant differences were observed between period of rapid development or rest period in these strains (Nestor et al., 1970). Thus, the egg type strain provided a better model for study since more eggs were available from individual hens compared to hens from other turkey strains. Birds were housed in separate cages and provided with good environmental conditions. Feed and water were ad libitum. The turkey laying ration provided a negative iodine balance to promote the excretion of free $^{125}$I after catabolism of $^{125}$I labeled proteins during the four experiments.

Nicholas white turkey poult s were obtained from a commercial hatchery within six hours after removal from the hatcher. The poult s were not treated with antibiotics. Birds were housed in a brooder
battery and provided with appropriate feed. Water supplemented with 0.01% potassium iodide was provided. The potassium iodide was added to promote the excretion of free \(^{125}\text{I}\) which was liberated from labeled IgG during the isotope experiment. Optimal environmental temperatures for growing turkey poult's were provided throughout the experiment.

It is important to note that in this experiment the birds' ages post-hatching can not be pinpointed. Turkey eggs are incubated for 28 days with hatching occurring within two days of the mean of 28 days. In this experiment it is possible that early hatching poult's were included in the experimental group, making possible ages as much as 2\(\frac{1}{2}\) days prior to injection.

**Immunodiffusion and immunoelectrophoresis**

Double immunodiffusion (ID) using 1% Noble agar in 0.05M sodium barbital buffer, pH 8.6 with 0.02% sodium azide was performed according to the method of Wadsworth (1957).

Immunoelectrophoresis (IEP) was performed in gels consisting of 1% Noble agar in 0.05M sodium barbital buffer, pH 8.6 with 0.02% sodium azide. Samples were run 90 minutes at 10 ma/frame in a Gelman electrophoresis apparatus (Saif et al., 1972).

**Chromatographic methods**

Sephadex G-200 was used in all gel filtration procedures. A series of two columns (2.5 x 45 cm and 2.5 x 100 cm) were used in the purification of IgG, IgM (Saif and Dohms, 1976), and IgA (Dohms et al., 1976). The column was equilibrated with borate buffered saline, pH 8.2 \(T/2 = .16\) (Benedict, 1968) and passed at a flow rate of 10 ml per hour, maintained by a peristaltic pump (Buchler Instruments, Fort Lee, N.J.).
Direction of flow was upward in the long column and downward in the short one. Fractions were recovered in volumes of 3 to 4 ml, and optical density (OD) was determined at 280 nm. A single column (2.5 x 45 cm) was used for gel filtration of plasma, egg yolk fractions and in the radioiodination procedures. In the latter procedure borate buffered saline, pH 8.2, containing 0.1% bovine serum albumin (Fraction V) and 0.02% sodium azide was used.

Final purifications of IgM, IgG, and egg yolk IgG were done by ion-exchange chromatography on diethylaminoethyl (DEAE) cellulose (Whatman DE 52) using stepwise buffer elution on a column of 1.5 x 30 cm at a flow rate of 15 ml/hr. The following stepwise changes in phosphate buffer were used: 1) 0.06M, pH 7.6; 2) 0.1M, pH 6.4; 3) 0.2M, pH 5.8; 4) 0.3M, pH 5.4; 5) 0.4M, pH 4.7; 6) 0.4M, pH 4.4, plus 2M NaCl. The stepwise Tris-HCl, NaCl buffer system of Watanabe et al. (1974) was used in the final purification of biliary IgA. This system consisted of stepwise elutions in 0.01M Tris-HCl buffer, pH 8.0 as follows: a) 0.05M NaCl, b) 0.1 M NaCl, c) 0.14M NaCl, d) 0.2 M NaCl, e) 0.3M NaCl, f) 0.5 M NaCl.

Isolation and purification of immunoglobulins

All turkey sera, fractions of sera, yolk and bile Ig preparations used in this study were maintained at 4 C. Serum was initially delipidated at 4 C with 0.04 ml of 5% dextran sulfate and 0.1 ml 0.25M MnCl₂ for each 1 ml of whole turkey serum (WTS) (Burstein et al., 1957). Centrifugation at 8,500 X g for 15 min at 4 C removed precipitated lipids. Isolation and purification of serum immunoglobulins M and G was by procedures similar to those described by Benedict (1968), using
Na₂SO₄ precipitations. Three successive salt precipitations at room
temperature of immunoglobulins were done from serum by the slow
addition of Na₂SO₄ to final concentrations of 0.18 g/ml, 0.14 g/ml, and
0.14 g/ml. After each precipitation the preparations were centrifuged
for 30 min at 524 X g, and the pellet was resuspended in one-half the
original serum volume in borate-buffered saline, pH 8.2. After the
final precipitation, resuspended immunoglobulins were dialyzed for 48
hr in borate-buffered saline at 4 C. The preparation was then filtered
on Sephadex G-200, producing two major peaks. The first peak
contained IgM, IgA, and traces of IgG and other minor contaminants.
The second peak fractions contained mostly IgG.

**Serum IgG purification**

The second peak fractions from Sephadex G-200, noted above, were
pooled and concentrated by ultrafiltration through an Amicon Diaflo
PM-30 membrane and recycled on columns until a single symmetrical peak
was observed on G-200 profile. IgM and other contaminants still could
be detected by ID after this step, so final purification was done by
DEAE chromatography. IgG was eluted at 0.06M PO₄ buffer, pH 7.6. IgG
at low concentrations could be recovered from 0.1M, 0.2M, and 0.3M
fractions, but other contaminants were also eluted in these areas and
therefore were discarded. IgM was observed in 0.2M, 0.3M, and 0.4M
fractions in small amounts. Recycling the 0.06M PO₄ fractions on DEAE
three times (reconcentrated approximately 12-fold after each DEAE
filtration) provided a purified IgG preparation that was filtered
through 0.45μ Millipore filters and stored at 4 C for future use.
Serum IgM purification

The ascending and the initial descending first peak fractions from several Sephadex G-200 filtrations of salt-precipitated immunoglobulins were refiltered on Sephadex G-200 until a symmetrical peak was observed. Fractions from the ascending part and the beginning of the descending part of the peak were pooled and saved. This pool contained IgM, IgA, and minor contaminants, such as IgG. Concentrated samples of the crude IgM pool were eluted on DEAE, and the fraction eluted by the .02M P0_4 contained most of the IgM. The samples were concentrated and recycled on DEAE until shown to be pure by immuno-diffusion testing. Recovery of IgM by the method described proved tedious, and the yield was relatively low. The purified IgM fractions were filtered through 0.45 Millipore filters and stored at 4 C.

Egg yolk IgG purification

Turkey IgG was also purified from turkey egg yolks. The water soluble fraction of yolks (WSF) was prepared by a modification of the procedure of Saito et al. (1965). One dozen egg yolks were freed of egg white by rolling on gauze and were diluted with an equal volume of phosphate buffered saline, pH 7.6 (PBS) and thoroughly mixed. Aliquots of this material were centrifuged at 33,750 X g (20,000 rpm on JA-20 Beckman head) for one hour at 18 C to pellet high density lipoprotein in the form of granules. The supernate was made 1M with 4M sodium chloride and recentrifuged at 141,650 X g (40,000 rpm on Beckman Sw-40 head) for 12 hours at 18 C. The bright yellow low density lipoprotein fraction (LDF) supernate was discarded leaving the clear WSF containing gammaglobulin.
The gammaglobulins were precipitated from the WSF using sodium sulfatate precipitations as described above for turkey serum globulins (Saif and Dohms, 1976). After the final precipitation the crude gammaglobulin was resuspended in borate buffered saline, pH 8.2 in one fourth the original yolk volume and dialyzed against four liters of the same buffer overnight at 6 C. Four to five milliliter aliquots of egg yolk globulins were subjected to G-200 gel filtration followed by passing the IgG containing peak through DEAE cellulose to obtain the .06M fraction for ID and IEP testing. The preparation was filtered through 0.45μ filters, and stored in sterile glass vials at 4 C until utilized for radiiodination or quantitative work.

**Biliary IgA purification**

Since the two IgA forms identified in turkey serum have not been purified, high molecular weight biliary IgA was utilized and purified in a manner previously described (Dohms *et al.*, 1976). Bile globulins were precipitated from freshly collected, heat inactivated turkey bile using two ammonium sulfate additions. Bile globulins were first precipitated by 50 percent saturated ammonium sulfate, followed by resuspension using distilled water. The final precipitation was accomplished by adding saturated ammonium sulfate so that one-half the final volume contained saturated ammonium sulfate.

Turkey IgA was purified from ammonium sulfate precipitated bile using Sephadex G-200 gel filtration followed by DEAE chromatography with stepwise elutions in .01M Tris-HCl buffer. IgA in bile was eluted on G-200 in the first peak, concentrated, then placed on DEAE columns where it eluted in the 0.3M NaCl fraction.
Purity of Ig preparations

Immunochemical purity of isolated Ig's was based on the following criteria: 1) a single line on ID and IEP against rabbit anti-whole-turkey serum (anti-WTS), and 2) lack of reactions against rabbit antisera to the heterologous Ig's.

Antisera production

Two rabbits were used for the production of each antisera reported. The production of these antisera were previously described (Saif and Dohms, 1976; Dohms et al., 1976).

Anti-whole-turkey serum

Each rabbit received a primary injection of 2 ml turkey serum mixed with an equal volume of Freunds' complete adjuvant. The mixture was administered intramuscularly (i.m.) at two different sites. Thereafter, each rabbit was given 1 ml serum without adjuvant every two weeks for two months, followed by one ml serum administered intravenously (i.v.). The rabbits were bled by heart puncture 7 days after the last injection.

Anti-IgM

Rabbit anti turkey IgM serum was prepared by a modification of the procedure described by Vaerman and Heremans (1972). *Salmonella heidelberg* O antigen, a thymus independent antigen (White and Nielsen, 1975), was inoculated i.v. into 30-lb male turkeys. On the fifth day post inoculation the birds were bled, and the serum was decomplemented with washed precipitates of bovine serum-anti bovine serum complexes prepared at antibody-antigen equivalence. Equal volumes of the preparation of O antigens, adjusted to McFarland 3, and the decomplemented turkey serum were mixed to absorb the specific IgM
antibodies. The resulting coated O antigen was washed three times by centrifugation at 3,100 X g using phosphate buffered saline, pH 7.4 to eliminate residual turkey serum in the preparation. One ml of the suspension was mixed with an equal volume of Freund's complete adjuvant for i.m. inoculation into each rabbit. Thereafter, 1 ml amounts of the suspension without adjuvant were inoculated following the schedule described for the production of anti-turkey serum. The serum was rendered monospecific (for μ chain) by absorption with small amounts of purified IgG.

**Anti IgG**

Approximately 1 ml purified IgG (1 ml) mixed with Freund's complete adjuvant was inoculated in the footpad of each rabbit. Three i.v. injections followed at two week intervals, approximately 1 mg IgG per inoculation, without adjuvant. Rabbits were bled out 7 days after the last injection, and the serum was rendered monospecific (for γ chain) by adsorption with small amounts of purified IgM or with an excess of Salmonella O antigen coated with IgM as described in the anti IgM preparations. Unabsorbed anti-IgG was used in quantitative work.

**Anti IgA**

Purified biliary IgA (approximately 1.0 mg/dose) was mixed with an equal volume of Freund's complete adjuvant and injected into the footpads of rabbits. At two week intervals i.m. injections were made with biliary IgA without adjuvant. Between two and four inoculations were made into each rabbit. Seven days after a final i.v. injection, rabbits were bled out. The serum was rendered monospecific for the α chain by absorption with IgM and IgG coated guinea pig erythrocyte
membranes. The absorption reagent was prepared from turkey serum obtained five days after injection of 1 ml of a 20% suspension of guinea pig erythrocytes. The following procedure was used for the preparation of this reagent:

1. Each one ml of washed guinea pig erythrocytes was packed at 310 x g for 15 minutes, and diluted with 10 ml of double distilled water in a centrifuge tube suitable for high speed centrifugation. After several minutes, membranes were pelleted by centrifugation at 48,000 x g for 30 minutes. The supernate was poured off leaving a pale reddish pellet.

2. Membranes were resuspended in 1 ml of .85% saline for each original one ml packed guinea pig erythrocytes and dispersed by passing the suspension through a 23 gauge needle connected to a 3.0 ml syringe.

3. One and one half ml of turkey anti guinea pig erythrocytes (5 day bleed out) were added to each ml of membrane-suspension, mixed, and incubated at 37 C for 60 minutes followed by overnight refrigeration at 4-6 C.

4. The turkey serum - guinea pig erythrocyte mixture was washed six times with 5 ml portions of normal saline then centrifuged at 480 X g for 15 minutes. After the last pelleting of the turkey IgM antibody coated membrane complex, the pellet was resuspended in 1 ml of rabbit anti turkey IgA and the pellet dispersed with a pasteur pipet. The mixture was incubated for one hour at 37 C and overnight at 4-6 C then spun at 480 X g to pellet the membrane complex. The supernate was finally tested for α chain monospecificity.
Antisera specificity

In the case of rabbit sera γ, μ, and α, the criteria used for antisera specificity were that the antisera would produce one precipitation line on ID or IEP against WTS and no visible reactions on ID or IEP against the heterologous Ig classes.

Iodination procedures

Two trace iodination techniques were utilized in different experiments using Iodine 125 (Amersham/Searle Corp., Arlington Heights, Ill.). One procedure utilized the lactoperoxidase technique to label turkey IgG (Marchalonis, 1969). The Chloramine-T technique was utilized to radioiodinate turkey Ig's in the other procedure (Hunter and Greenwood, 1962).

In the lactoperoxidase labeling technique, twenty μg of turkey IgG in 80 μl .06M phosphate buffer, pH 7.6 was diluted with 40 μl of 0.4M acetate buffer, pH 5.6 in a 6 x 50 mm glass reaction vial. Bovine lactoperoxidase, 0.4 I.U. in 80 μl .1M acetate buffer pH 5.6 and 50 μCi of I\(^{125}\) in 0.5 μl of NaOH (Amersham/Searle IMS 30) was added to the reaction vial with mixing. The iodination reaction was initiated at room temperature by adding 10 μl of hydrogen peroxide (3% freshly diluted 1:1500) and the reaction terminated after five minutes by the addition of 100 μl of 16% sucrose with 1% potassium iodine and .02% sodium azide. The reaction mixture was subjected to G-200 gel filtration and the eluted fractions were collected in four milliliter aliquots and counted for radioactivity on an automated gamma counter (1185 series, Nuclear-chicago, Des Plaines, Ill.). The protein bound radioactivity eluting in the first radioactivity peak was collected,
pooled, and dialyzed against three changes totaling six liters of PBS at 6°C to remove residual free iodine. An aliquot of this material was counted to determine specific activity and re-filtered on G-200 to evaluate the removal of free \(^{125}\text{I}\) from protein bound \(^{125}\text{I}\).

In the chloramine-T procedure, purified Ig preparations in .06M phosphate buffer, pH 7.6, were diluted so that 25-75 µg of Ig in 50 µl of the same buffer was placed in a 6 x 50 mm reaction vial in an ice bath. Seventy µCi \(^{125}\text{I}\) contained in 1 µl dilute sodium hydroxide was added to the reaction tube. Freshly prepared chloramine-T at 60 µg in 20 µl of .25M phosphate buffer, pH 7.5 was added to the reaction tube with vigorous mixing for two and one half minutes. The addition of 125 µg of sodium metabisulfite in 50 µl of .25M phosphate buffer, pH 7.5 terminated the reaction. A 16% sucrose solution was added to increase the density of the solution and the contents of the reaction vial were subjected to G-200 gel filtration. The remaining steps in preparation of labeled protein were identical to the methods used in the above lactoperoxidase technique.

**Radial immune diffusion assay**

Plasma and egg yolk IgG levels in adult turkey hens and in young pouls were determined by a modification of the radial immune diffusion assay (RID) of Heremans (1971). Stock IgG solutions were quantitated using the method of Lowrey (1951). Standards of 20, 100, 150, and 200 µg IgG/ml were prepared by dilution of stock IgG in .05M sodium barbital buffer, pH 8.6 with .025% sodium azide. The RID assay was conducted in gels consisting of 1.5% Noble agar containing 1% rabbit anti turkey IgG in .05M sodium barbital buffer, pH 8.6 and .025% sodium
azide that were prepared in 6 x 11 cm immunodiffusion plates (Miles Laboratories, Inc., Elkhart, Ind.).

Radial immune diffusion gels were prepared from 2x concentrated stock solutions of Nable agar (3%) and antisera (2%) prepared in sodium barbital-azide buffer. Agar stock was melted in a boiling water bath and placed with freshly prepared antiserum stock into a 58 C water bath until both solutions reached 58 C. Equal volumes of agar and antiserum were combined and slowly mixed with a preheated 10 ml pipet in the water bath. Twelve ml aliquots were removed by pipet and poured onto ID plates with care taken to prevent the formation of bubbles. Plates were poured on a level surface so that uniform depth was achieved throughout each agar plate. After cooling and agar solidification, holes were punched and removed using a 17 well template with .2 cm diameter gel punch provided with negative pressure. Duplicates of the standards were used on each plate along with triplicate plasma and yolk WSF samples diluted in barbital buffer. All samples and standards were applied in 10 µl amounts using a 50 µl Hamilton microsyringe.

Gels were incubated for five days at room temperature under a layer of mineral oil before being photographed. Photographic enlargements (20 x 26 cm) of plates allowed measurement of the diameters of each precipitin ring. Standard curves were constructed for each plate allowing the determination of IgG concentrations in unknown samples. The standard curves were linear (r > .95) from 20 - 200 µg/ml with the lower limit of sensitivity at 20 µg/ml.
Preparation of egg yolk for quantitative work

The egg yolk WSF prepared by the procedure described above was utilized for quantitating IgG levels in egg yolks from individual hens. Dilutions were recorded allowing correction after assays.

Evaluation of percent recovery of turkey IgG from egg yolk

In order to quantitate IgG levels in egg yolk, a procedure was designed for evaluating the percent recovery of IgG in the WSF of egg yolk using the preparative procedures outlined in the previous section. Eggs were obtained from hens injected with labeled IgG that had substantial radioactivity associated with egg yolk. The granules, LDF, and WSF of five individual egg yolks were counted during WSF preparation allowing calculation of the average overall recovery and the recovery at each step during fractionation. Percent recovery data allowed correction of the results of IgG quantitation in the WSF using the RID assay.

Blood sampling and handling

Blood samples were obtained from the wing veins of hens in the radioisotope experiments using heparinized 5 ml syringes with 23 gauge needles. For repeated sampling, samples were drawn from alternate wing veins.

Blood plasma was recovered after pelleting erythrocytes by centrifugation at 310 X g for 15 minutes and stored at 6 C in glass vials until radioactivity counting or quantitative procedures were completed. For plasma volume determinations, packed cell determinations were conducted on aliquots of samples collected into capillary tubes. The tubes were then sealed and centrifuged for 15
minutes at 310 X g using a microhematocrit centrifuge (International Equipment Co., Boston, Mass.) before the packed cell volumes were determined.

Blood samples were obtained individually and repeatedly from pouls by puncturing the wing vein. Blood was collected in two to three heparinized capillary tubes at the site of puncture. Individual hematocrit values were obtained for each bird at sampling intervals using the microhematocrit system described above. The capillary tubes were then scored, broken at a point adjacent to the packed cells and the plasma was recovered on parafilm (Parafilm M, American Can Co., Neeah, Wis.). Between 5 and 150 µl of plasma was obtained using Kimball microcapillary pipets for further procedures and assays.

If temporary storage was required, aliquots were diluted with an equal volume of .05M sodium barbital buffer with .025% sodium azide and stored in sterile 6 x 50 mm glass tubes.

Yolk sac absorption in pouls

Twenty-eight turkey pouls were obtained within 6 hours of removal from the hatcher. Birds were housed in a brooder and provided with appropriate feed and water ad libitum. At twelve hours after removal from the hatcher ten pouls were sacrificed and whole body and yolk sac weights were determined. The procedure was repeated using six pouls at 1, 2, and 3 days after removal from the hatcher. This experiment provided data on absorption of the yolk sac in the turkey poult.

Calculations and statistical analysis

The following calculations were made:
1) Efficiency of gamma counter using a prepared $^{125}\text{I}$ standard = counts per minute (cpm) standard - background (bkg) cpm \div known disintegrations per minute (dpm) for standard \times 100.

2) The dpm of a counted standard = cpm - bkg \div efficiency.

3) The protein specific radioactivity at times after injection was corrected for decay rate of $^{125}\text{I}$, protein associated dpm = dpm \div the percent of original $^{125}\text{I}$ remaining on the day counted.

4) The blood volume was determined in turkey poults assuming the whole blood volume to be ten percent of whole body weight. In hens, blood volume was determined by isotope dilutions from the intercept of the fast decaying slope of radioactivity decay.

5) Plasma volume = blood volume (ml) - (blood volume \times mean percent hematocrit values).

6) The labeled Ig in plasma pool = dpm/ml \times plasma volume.

7) The percentage of the Ig distributed into the intravascular space was determined from the dpm at the intercept of final linear decay slope \times the plasma volume \div total injected dose \times 100.

8) Fractional turnover rate (K days$^{-1}$) which is the first order rate constant for labeled Ig = the slope of the final calculated linear regression line (b) \times 2.302. The constant K \times 100 = the Ig turnover expressed as percent lost from the plasma pool per day.

9) The half-life of Ig in days ($t^{1/2}$) = .693 \div K.

10) The total turnover rate of Ig in the body in mg lost/day = K \times M where M is the plasma pool IgG concentration.

All radioactive samples were counted on an automated gamma counter so that statistical precision on accumulated counts gave a 2.5%
deviation at a 99.73% confidence interval. Radioactivity time curves were resolved into one or two exponential functions using linear regression and "peeling" procedures (Shipley and Clark, 1972). Linear regression and one-way analysis of variance involved use of programs for a Monroe model 1760 desk top calculator. Least Square Means analysis of variance with covariate analysis utilized a computer program.
PROCEDURES AND RESULTS

The Metabolism and Passive Transfer of Immunoglobulins in the Turkey Hen

Experiment 1 - The metabolism of IgG in turkey hens.

The experiment was designed to study the overall metabolism of IgG in the turkey hen as well as to reveal the kinetics of passive transfer of IgG to egg yolk and white. Four turkey hens were weighed and each was injected intravenously with 2 µCi 125I labeled IgG (4.4 X 10^6 dpm) (Table 3). Whole blood samples were drawn in heparinized syringes at .6, 2.5, and 6 hours post-injection from the alternate wing vein. Blood samples were drawn daily until 4 days post-injection, thereafter longer intervals between bleedings occurred until 32 to 34 days after injection. One milliliter aliquots of whole blood were counted on an automated gamma counter to determine the dpm/ml whole blood. Plasma IgG concentration from each hen was measured using the RID assay which allowed calculation of the plasma pool size for IgG. Eggs were collected daily at appointed times and individually measured volumes of diluted mixed egg yolks and undiluted egg white were prepared. Egg fractions were counted and plotted to indicate the accumulation and decline of radioactivity during the experiment. The association of radioactivity with IgG in plasma and egg fractions was evaluated using either G-200 chromatography or precipitin of protein
Table 3. Summary of some data of experiments one through 4 showing the individual hen weights, labeled protein, labeling technique, specific activity, total activity of injected dose, and route of injected label.

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Hen no.</th>
<th>Wt. (kg)</th>
<th>Labeled protein</th>
<th>Iodination method</th>
<th>Specific activity (µCi/µg)</th>
<th>Route of injection</th>
<th>Injected dose (DPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>7.35</td>
<td>IgG</td>
<td>Bov. LacPerox.</td>
<td>.20</td>
<td>i.v.</td>
<td>4.41x10^6</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>6.44</td>
<td>IgG</td>
<td>Bov. LacPerox.</td>
<td>.20</td>
<td>i.v.</td>
<td>4.41x10^6</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>7.03</td>
<td>IgG</td>
<td>Bov. LacPerox.</td>
<td>.20</td>
<td>i.v.</td>
<td>4.38x10^6</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>6.85</td>
<td>IgG</td>
<td>Bov. LacPerox.</td>
<td>.20</td>
<td>i.v.</td>
<td>4.41x10^6</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>6.35</td>
<td>IgG</td>
<td>Chloramine T</td>
<td>.44</td>
<td>i.v.</td>
<td>6.66x10^6</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>7.26</td>
<td>IgG</td>
<td>Chloramine T</td>
<td>.44</td>
<td>i.v.</td>
<td>6.66x10^6</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>6.58</td>
<td>IgM</td>
<td>Chloramine T</td>
<td>.17</td>
<td>i.v., i.p.</td>
<td>3.86x10^6</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>7.48</td>
<td>IgM</td>
<td>Chloramine T</td>
<td>.17</td>
<td>i.v.</td>
<td>3.86x10^6</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>6.80</td>
<td>IgA, biliary</td>
<td>Chloramine T</td>
<td>.10</td>
<td>i.v.</td>
<td>2.22x10^6</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>6.58</td>
<td>IgA, biliary</td>
<td>Chloramine T</td>
<td>.10</td>
<td>i.v.</td>
<td>1.9x10^6</td>
</tr>
</tbody>
</table>
bound radioactivity with 10% trichloroacetic acid (TCA). The WSF of representative eggs sampled during the experimental period were assayed for IgG concentration using RID assay.

A G-200 chromatograph of an aliquot of labeled IgG appears in Fig. 1. Analysis of this chromatograph showed that 98% of the radioactivity was associated with IgG.

To insure that radioactivity was associated with IgG in vivo, representative G-200 chromatographs of plasma and egg yolk WSF are presented in Fig. 1. Examination of chromatographs of plasma and WSF of yolk revealed IgG associated radioactivity for all four hens sampled at times near injection and at a later point in the final linear decay slope. Radioactivity of egg white was too low to be detected using gel filtration but aliquots of sampled egg white had radioactivity (1 to 3 days) after injection and was precipitated with an equal volume of ice cold 10% trichloroacetic acid. Only 53.3±1.3% of the radioactivity of peak times after injection was TCA precipitable and was assumed to be IgG.

The IgG specific radioactivity time curve appears in Fig. 2. Results from one hen are presented and were similar to those from other hens in this experiment. Statistical peeling procedures revealed that the injected dose was distributed into two pools as indicated by different exponential rates of elimination. The results of calculations of fractional turnover rate, half-life, total turnover rate, and percent turnover from each hen appear in Table 4.

Two components (fast and slow decaying) were observed. The initial "fast" decaying component with a steep slope had a mean
Figure 1. Sephadex G-200 chromatographs of (A) injected labeled IgG, (B) plasma 15 minutes after injection of labeled IgG, (C) water soluble fraction of egg yolk after IgG injection, (D) an aliquot of injected labeled IgM, (E) plasma from a hen after injection of labeled IgM, (F) an aliquot of injected labeled IgA, (G) hen plasma after injected with labeled IgA.

The solid lines represent the optical density readings at 280 nm and the broken lines represent specific radioactivity.
Figure 2. The IgG, IgM, and IgA specific radioactivity time curves in turkey hens.

Open and closed circles represent data points from different birds.
half-life of 1.07±.14 days. The "slow" decaying component had a mean half-life of 5.91±.41 days and 40% was distributed to the intravascular space (range 26-52%). The plasma IgG concentration determined from plasma samples using RID assays were used in turnover rate calculations. The plasma IgG concentration ranged from 7.30 - 10.04 mg/ml with a mean of 8.54±.59 mg/ml and the total calculated plasma pool having a mean of .518±.07 grams IgG/kilogram body weight of adult hen. Assuming steady state kinetics (IgG catabolism equals synthesis) and that the "slow" slope represents the half-life of IgG, the total body turnover rate of .062±.01 gm/kg/day is irreversibly lost to endogenous catabolism or into egg material. This total loss represents 11.92±.01% of the plasma pool IgG lost per day.

Data on transfer of labeled IgG into egg yolk and white in turkey hens 1 through 4 appear in Fig. 3. Whole blood, egg yolk and egg white were plotted as dpm/ml material throughout the experiment. The first egg layed within 24 hours after injection had little or no radioactivity; however, increasing amounts of label were observed until peak levels were attained at 6.5±.7 days post injection corresponding to the sixth to eighth egg layed after injection. Thereafter, the decay curve of egg yolk declined and later paralleled the whole blood decay curve. This is particularly evident with hen 1 that had fewer pauses in laying during the experiment. Statistical analysis comparing slopes of whole blood and egg yolk decay in hens that had fewest laying pauses (hen 1 and 2) showed slopes, k values and half-lives that were nearly identical. This parallelism occurring
Figure 3. The distribution of labeled IgG in whole blood, egg yolk, and egg white in four turkey hens during a thirty-four day period following injection.
Table 4. The IgG fractional turnover rate, half-life, plasma concentration, total plasma volume, total IgG plasma pool, and total IgG turnover rate for four laying turkey hens.

<table>
<thead>
<tr>
<th>Hen no.</th>
<th>Fractional turnover rate (k)</th>
<th>Half-life days</th>
<th>Plasma IgG concentration mg/ml</th>
<th>Plasma volume ml/kg</th>
<th>IgG plasma pool gm/kg</th>
<th>Total turnover rate gm/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;fast&quot; 0.835</td>
<td>0.83 digits</td>
<td>7.30</td>
<td>45.1</td>
<td>0.329</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>&quot;slow&quot; 0.1157</td>
<td>0.99 digits</td>
<td>10.04</td>
<td>56.3</td>
<td>0.566</td>
<td>0.083</td>
</tr>
<tr>
<td>2</td>
<td>&quot;fast&quot; 0.98</td>
<td>4.75 digits</td>
<td>0.98</td>
<td>0.751</td>
<td>0.045</td>
<td>0.073</td>
</tr>
<tr>
<td>3</td>
<td>&quot;slow&quot; 0.98</td>
<td>6.29 digits</td>
<td>0.98</td>
<td>0.83</td>
<td>0.665</td>
<td>0.073</td>
</tr>
<tr>
<td>4</td>
<td>&quot;fast&quot; 1.49</td>
<td>6.59 digits</td>
<td>0.81</td>
<td>0.57</td>
<td>0.510</td>
<td>0.054</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>8.54</td>
<td>60.7</td>
<td>0.518</td>
<td>0.062</td>
</tr>
</tbody>
</table>

a K is expressed in reciprocal days. The fractional turnover rate X 100 equals the turnover rate as percent of the plasma pool lost per day.

b Calculated by dilution of the injected label using the intercept of the fastest decaying component.
after equilibration suggests that in individual hens IgG is transferred to ova at a constant rate.

Although the radioactivity in egg whites was shown to be only 53.3±1.3% TCA precipitable at peak levels, it is interesting to note that radioactivity in white peaked at 1.75±.5 days, 4.75 days earlier than observed peaks in egg yolk.

The evaluation of percent recovery of IgG in the WSF showed that the overall recovery of 62.9±3.1% was observed (range 51.49-69.96%). The results of RID assays corrected for percent recovery on egg yolk WSF samples from three hens during the experiment appear in Table 5. Graphic analysis showed that the overall trend in IgG level during the experiment was linear with average slopes of .00062 indicating that no upward or downward trend in IgG transfer occurred. Random variation around these lines was believed to reflect the variations in percent recovery, and RID assay.

Significant differences were seen between hens for IgG concentration and in total IgG per ova with hen 2 being significantly higher than the other hens tested (p < .005). Corrections for individual egg weight differences by analysis of covariance indicated that hen 2 still had significantly higher levels of IgG in egg yolks (p < .007).

**Experiment 2 - The rate of IgG transfer to developing ova.**

Further information on the rate of transfer of IgG to ova was revealed by using short term exposure to injected labeled IgG followed by counting the radioactivity in representative smaller ova and all ova in rapid development. Two hens were injected intravenously with
Table 5. IgG content of selected yolks from three hens sampled during the 34-day experimental period.

<table>
<thead>
<tr>
<th>Egg no.</th>
<th>Hen 1</th>
<th>Hen 2</th>
<th>Hen 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG conc. mg/ml</td>
<td>Total IgG/ova mg</td>
<td>IgG conc. mg/ml</td>
</tr>
<tr>
<td>1</td>
<td>2.1</td>
<td>47.2</td>
<td>4.0</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>72.8</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>3.2</td>
<td>72.7</td>
<td>3.8</td>
</tr>
<tr>
<td>4</td>
<td>2.9</td>
<td>75.3</td>
<td>4.3</td>
</tr>
<tr>
<td>5</td>
<td>2.8</td>
<td>66.9</td>
<td>5.1</td>
</tr>
<tr>
<td>6</td>
<td>4.0</td>
<td>98.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Mean ± σ</td>
<td>3.0±0.3</td>
<td>72.1±6.7</td>
<td>4.1±0.3^a</td>
</tr>
<tr>
<td>Adjusted mean ± σ^b</td>
<td>76.4±7.1</td>
<td>102.3±6.1^a</td>
<td>73.3±6.0</td>
</tr>
</tbody>
</table>

^aStatistically significant critical value for F distribution compared to corresponding means for eggs of other hens.

^bMeans adjusted for individual egg weight differences by analysis of covariance.
3 μCi $^{125}$I labeled IgG, $6.6 \times 10^6$ dpm (Table 3). Twenty-four hours later the hens were sacrificed and the ova were removed from the ovaries of each bird. All ova were freed of capillary beds in the outside membranes, weighed, measured, diluted with an equal volume of physiological saline and counted for radioactivity.

The rate of transfer of $^{125}$I labeled IgG by individual ova over a 24-hour period appear in Fig. 4. The total $\log_{10}$ dpm/ova plotted according to individual ova weight (dose adjusted for differences in hen weight) indicated that a rapid transfer of IgG occurred as the ova developed from .6 - 1.0 gm weight (approximately 10 mm diameter) corresponding to the beginning of the rapid development phase of oocyte growth. Thereafter, ova had increasing amounts of radioactivity throughout the middle size range followed by a decline in the rate of transfer in the two or three largest ova. In order to remove the differences in ova weights observed between hens, and to analyze IgG transfer in relation to surface area, the same data was plotted in Fig. 4 as $\log_{10}$ dpm/cm$^2$ ova on the ordinate, and ova weight expressed as percent of total development on the abscissa. The observed differences in IgG transfer between hens were removed by adjusting for ova weight. In addition, in these two hens, each square centimeter of follicular epithelium appears to transfer IgG at about the same rate during corresponding stages of ova development. The middle size ova have a slightly higher uptake than the early and later periods of development. The higher levels of radioactivity in the middle range (40 - 60% of total development) are more apparent when the data was
Figure 4. The rate of transfer of labeled IgG in a 24-hour period in individual ova recovered from the ovaries of two turkey hens.

On the left, the total accumulated radioactivity in different size ova is presented. On the right the same data is presented as accumulated radioactivity per unit surface area (log dpm/cm²) in the different ova adjusted so that weight is expressed as the percent of full development.
plotted without logarithmic transformation of dpm/cm² ova surface (not shown).

**Experiment 3 - The transfer and half-life determinations of IgM.**

In order to determine if IgM was transferred to eggs and to obtain data on the metabolism of this protein in laying hens, an experiment was designed similar to experiment 1 except that two hens were each injected with labeled IgM (Table 3). In addition, blood plasma instead of whole blood radioactivity was counted to determine specific Ig radioactive decay. Quantitative determinations were not attempted on plasma or egg yolk material in this experiment.

A Sephadex G-200 chromatograph of an aliquot of labeled IgM appears in Figure 1. It was determined that 100% of the radioactivity was associated with IgM.

Sephadex gel filtration of plasma samples, one and two days post-injection indicated that radioactivity was associated with IgM protein in vivo (Fig. 1). The plasma radioactivity time curve for IgM injected birds dropped fast, and it was not detectable after G-200 filtration of plasma samples at later periods along the decay curve. In order to demonstrate that radioactivity was associated with protein at these times, larger volumes (20 ml/bird) of pooled plasma samples from individual birds were precipitated with equal volumes of cold 10% TCA. After extensive washing with additional TCA, the samples were counted and the radioactivity was found exclusively associated with the precipitated protein.

G-200 chromatographs of yolk WSF in which appreciable amounts of radioactivity were found indicated that the radioactivity was not
associated with the IgM peak but chromatographed at a point of total column volume. Because only a limited volume of sample could be placed on a G-200 column, it was decided to precipitate 25 ml of yolk plasma with 10% TCA from the points of observed peak radioactivity. The precipitates were washed with about 300 ml of TCA and counted for radioactivity. About 200 dpm above background was observed in 2 trials suggesting that very little or no labeled IgM was transferred into egg yolks. Precipitin lines were not observed using rabbit anti-\( \mu \) serum following I.D. assay of yolk material that had been tenfold concentrated by ammonium sulfate precipitation. Radioactivity in egg whites was too low to be used effectively for examination of association with IgM. However, I.D. results using undiluted egg white revealed precipitin lines in 21% of 30 eggs examined using rabbit anti-\( \mu \) sera.

The IgM specific radioactivity time curve presented in Fig. 2 shows the elimination of IgM from hens 7 and 8. In this experiment it was difficult to evaluate if the injected dose was distributed into more than one pool. This in part was due to sampling procedures which were hampered by extensive blood hematomas at the time of injection and early in the sampling period. Nevertheless, the final linear slope allowed the calculation of half-life of IgM of 2.69 and 2.74 days, respectively (Table 6). This represents IgM turnover of 25.76 and 25.29% of the plasma pool per day.

**Experiment 4 - The transfer and half-life determinations of IgA.**

In order to determine if IgA was transferred to eggs and to obtain data on the metabolism of this protein in laying hens, this
Table 6. The fractional turnover rate and half-life of IgM and biliary IgA in laying turkey hens.

<table>
<thead>
<tr>
<th>Hen no.</th>
<th>Labeled protein</th>
<th>Fractional turnover rate (k)\textsuperscript{a}</th>
<th>Half-life (t\textsuperscript{52})</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>IgM</td>
<td>.2576</td>
<td>2.69</td>
</tr>
<tr>
<td>8</td>
<td>IgM</td>
<td>.2529</td>
<td>2.74</td>
</tr>
<tr>
<td>9</td>
<td>IgA</td>
<td>.3609</td>
<td>1.92</td>
</tr>
<tr>
<td>10</td>
<td>IgA</td>
<td>.4125</td>
<td>1.68</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The fractional turnover rate X 100 equals the turnover rate expressed as percent of the plasma pool lost per day.
experiment was designed similar to experiments 1 and 3 except that two hens were each injected with labeled IgA (Table 3). In addition, blood plasma instead of whole blood radioactivity was counted to determine specific Ig radioactive decay. Quantitative determinations were not attempted on plasma or egg yolk material in this experiment.

A Sephadex G-200 chromatograph of an aliquot of labeled IgA appears in Figure 1. It was determined from the chromatograph that 100% of the radioactivity was associated with IgA.

Sephadex G-200 gel filtration results are similar to results reported for IgM injected hens. Radioactivity was exclusively found in the plasma at 1 to 2 days after injection and later TCA precipitates revealed radioactivity associated with protein in the final decay slope.

Radioactivity in egg yolk material was substantial but associated with free iodine when examined using G-200 chromatography. A small amount of protein associated radioactivity (about 200 dpm) was found by precipitating large (25 ml) volumes of yolk with TCA followed by extensive washing. Therefore like IgM, little or no IgA was detected in unincubated egg yolk. Likewise, I.D. results of yolk material concentrated tenfold by ammonium sulfate precipitation were negative using rabbit anti-α sera. Levels of radioactivity in unincubated egg whites were too low to effectively examine using either G-200 or TCA precipitation. However, no IgA was detected in undiluted egg whites using I.D. assay.

The IgA specific radioactivity time curve presented in Fig. 2 shows the elimination of IgA from hen 9. A similar pattern was
observed in hen 10. The injected IgA was distributed into two compounds. An extremely steep initial decay line indicates rapid removal of the labeled protein from the blood plasma with a half-life of 3 hours. A slower final decay slope had a half-life of 1.92 and 1.68 days in hens 9 and 10 (Table 6).

Studies on the Metabolism and Levels of Immunoglobulin G in the Newly Hatched Turkey Poult

Experiment 5 - Metabolism of IgG in Turkey Poult

This experiment examined the question of how long natural IgG exists in the young poult and its contribution to the levels of IgG during early growth in this species.

Seven poultls were individually weighed within twelve hours of being removed from the incubator, injected intra-abdominally with 1.2 μCi (2.66 X 10^6 dpm) of labeled IgG and placed in the brooder. Blood samples were collected from each bird on days 1, 3, 6, 8, 10, 13, 17 and 21 after injection. At each sampling date birds were individually weighed, and hematocrit values and blood and plasma volumes were calculated. Radioactivity counts and RID assays on plasma aliquots collected from each poult during the three-week period of the experiment allowed the calculation of the dpm of injected dose of IgG per ml plasma, dpm of injected dose of IgG in the total plasma pool, concentration of IgG per ml of plasma and concentration of IgG in total plasma pool.

On day 14 post-injection one bird appeared clinically ill, and was removed from the study. Cultural examination indicated a Salmonella infection. One other bird was removed from the study at day
because of weight loss associated with dehydration accompanying a Salmonella infection. It is possible that the other birds were infected with Salmonella without exhibiting clinical signs. However, in these birds growth rates were unaffected when compared to normal growth patterns for young turkeys.

A G-200 chromatograph of an aliquot of the injected dose of labeled IgG appears in Fig. 5. A single peak, co-chromatographing in a position identical with purified IgG indicated that no observable gross alterations or denaturation took place during iodination. A small amount of free iodine was observed to elute in a position at total column volume. This free iodine constituted 2% of the injected dose. To insure that the observed plasma radioactivity was associated with labeled IgG throughout the experiment a pooled sample of plasma from one bird collected at 3, 6, 8, 10 and 13 days post-injection (PI) was chromatographed on G-200 gel (Fig. 5) and the radioactivity was observed to co-chromatograph in a position identical to purified IgG and was exclusively associated with this peak.

The mean and standard error of radioactivity values for all poultts during the 21-day study are presented in Fig. 6. Data plotted for all individual birds had a similar pattern. Equilibration between intravascular and extravascular pools required between four and six days followed by the linear decay curve observed in logarithmically transformed data. The slope of the final total plasma pool radioactivity line is not as steep as the slope of the decay curve on a fixed one milliliter volume of plasma since the total plasma pool
Figure 5. Sephadex G-200 chromatographs of an aliquot of (A) injected labeled IgG, and (B) pooled plasma sample from days 3 through 13 post-injection. Symbol (±) indicates the elution position of purified unlabeled IgG.
Figure 6. IgG specific radioactivity in the blood plasma of turkey poults after injection of labeled IgG.

Mean and standard errors of total log dpm in plasma pool (▲) and log dpm per milliliter plasma (●) are plotted at sampling times during the experiment.
curve corrects for the dilutional effect of increasing plasma volume during growth.

The calculated half-life of IgG in individual pouls per milliliter of plasma appears in Table 7. The correlation coefficients (r) for the linear regressions indicated good correlation for linearity (r > .9) using points after equilibration, days 3 or 6 to 21. A mean half-life for IgG of 2.85±.098 days was calculated.

Calculations of the whole plasma pool decay of labeled IgG showed a half-life of 4.08±.33 days. Data on individual pouls appear in Table 8. Again, good correlation coefficients for the regressions (> .9) were observed on data points from days 7 through 21. Calculated total body IgG turnover per day in milligrams from days 6 through 21 PI appears in Table 3. Thus, the turnover rate (amount of IgG lost/day) was directly correlated with the plasma pool concentrations of IgG, but the total irreversible loss for all pouls was very similar from days 6 to 21.

By extending the calculated regression line in the plasma pool to the time of injection (the y intercept), a theoretical value of the total labeled IgG in plasma pool at time of injection (T1) was made for each bird. By designating the mean value of the intercepts at T1 as 100% plasma level at this time, percentages of this value at day 6, 8, 10, 13, 17, and 21 were calculated. After logarithmic transformation, these data are linear as indicated by a .994 correlation coefficient for linear regression. Fig. 7 presents the data on elimination of the injected dose with the calculated means, standard errors and a 95% confidence interval for the regression line.
Table 7. The slope, correlation coefficient of regression, fractional turnover rate and half-life of egg yolk IgG in the plasma of turkey poults determined from a fixed one milliliter volume of blood plasma.

<table>
<thead>
<tr>
<th>Poultno.</th>
<th>Slope of plasma activitya</th>
<th>Linear correlation (r)b</th>
<th>Fractional turnover rate (k, days⁻¹)</th>
<th>Half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.099</td>
<td>.999</td>
<td>.230</td>
<td>3.01</td>
</tr>
<tr>
<td>2</td>
<td>.103</td>
<td>.996</td>
<td>.237</td>
<td>2.93</td>
</tr>
<tr>
<td>3</td>
<td>.109</td>
<td>.991</td>
<td>.250</td>
<td>2.77</td>
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<tr>
<td>4</td>
<td>.110</td>
<td>.997</td>
<td>.252</td>
<td>2.75</td>
</tr>
<tr>
<td>5</td>
<td>.104</td>
<td>.986</td>
<td>.239</td>
<td>2.90</td>
</tr>
<tr>
<td>6</td>
<td>.093</td>
<td>.996</td>
<td>.214</td>
<td>3.23</td>
</tr>
<tr>
<td>7</td>
<td>.126</td>
<td>.995</td>
<td>.291</td>
<td>2.39</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>2.85</td>
</tr>
<tr>
<td>σ</td>
<td></td>
<td></td>
<td></td>
<td>.098</td>
</tr>
</tbody>
</table>

aThe slope of the IgG specific radioactivity time curve is defined by the formula $y = bx + a$ where $b$ is the slope, $y$ is $\log_{10}$ dpm, $x$ is days, and $a$ is the $y$ intercept.

b$r$ = correlation coefficient for calculated linear regression.
Table 8. The slope, correlation coefficient of regression, fractional turnover rate, and half-life of egg yolk IgG in the total plasma pool of turkey poult determined from day 6 through 21 after labeled IgG injection.

<table>
<thead>
<tr>
<th>Poult no.</th>
<th>Slope of plasma activity(^a)</th>
<th>Linear correlation (r)(^b)</th>
<th>Fractional turnover rate (k, days(^{-1}))</th>
<th>Half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-.061</td>
<td>.985</td>
<td>.140</td>
<td>4.95</td>
</tr>
<tr>
<td>2</td>
<td>-.083</td>
<td>.993</td>
<td>.190</td>
<td>3.64</td>
</tr>
<tr>
<td>3</td>
<td>-.082</td>
<td>.991</td>
<td>.189</td>
<td>3.67</td>
</tr>
<tr>
<td>4</td>
<td>-.078</td>
<td>.998</td>
<td>.180</td>
<td>3.85</td>
</tr>
<tr>
<td>5</td>
<td>-.088</td>
<td>.996</td>
<td>.201</td>
<td>3.44</td>
</tr>
<tr>
<td>6</td>
<td>-.053</td>
<td>.987</td>
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<tr>
<td>7</td>
<td>-.090</td>
<td>.998</td>
<td>.207</td>
<td>3.35</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>4.08</td>
</tr>
<tr>
<td>(\sigma)</td>
<td></td>
<td></td>
<td></td>
<td>.332</td>
</tr>
</tbody>
</table>

\(^a\)The slope of the IgG specific radioactivity time curve is defined by the formula \(y = bx + a\) where \(b\) is the slope, \(y\) is \(\log_{10}\) dpm, \(x\) is days, and \(a\) is the intercept.

\(^b\)\(r\) = correlation coefficient for linear regression.
Figure 7. Decay of injected labeled IgG from the plasma of turkey poults expressed as percent of remaining injected dose. Experimentally calculated means and standard errors are presented with a 95% confidence interval for the regression line.
By day seven PI 30.53% of the injected dose remained in the plasma pool; by 14 days PI 10.31% remained, and by 21 days PI only 3.48% of the injected dose remained in the plasma pool.

Mean values of IgG concentration in mg/ml plasma as calculated from the results of RID assay appear in Fig. 8. Analysis of variance testing revealed no significant difference between mean values of IgG concentrations of 0, 1, and 3 days PI or between means of days 8 through 21. A significant drop was observed from days 4 through 6 (p < .05) and from days 6 through 8 (p < .05).

A more accurate measure of IgG levels in poultis is seen in the calculated total plasma pool IgG in Fig. 8 and Table 9. Although a slight increasing trend is observed from days 0 through 6, this is not significantly different (p < .25). A significant increase was observed from day 10 through 13 (p < .001) and from days 17 through 21 PI (p < .001).

It was possible to calculate the catabolism of the total plasma pool IgG at time of injection based upon the data for radioactive decay of the injected labeled IgG. The data in Fig. 8 present the maternal decay curve in milligrams plasma pool IgG as calculated from the curve of iodine\(^{125}\) labeled IgG decay; Fig. 7 using the mean of the total IgG pool at time of injection (T\(_1\)) (Table 9). In this curve standard errors are not presented. However, it is recognized that the standard errors of both the IgG plasma pool concentration at T\(_1\) and the standard error of the regression line (Fig. 7) would provide variation around this idealized curve. This curve is similar to the decay of maternal IgG assuming that all the plasma IgG at T\(_1\) is of maternal origin. The
Figure 8. (A) The mean and standard errors of IgG concentrations in mg/ml plasma from turkey poultts. (B) The total mean and standard errors of plasma pool IgG levels in turkey poultts. The broken line indicates the decay of the total IgG plasma pool present at time of injection.
Table 9. IgG plasma pool levels and total turnover rate (total irreversible loss) of IgG in turkey poult:s determined after injection of labeled IgG.

<table>
<thead>
<tr>
<th>Day</th>
<th>IgG plasma pool conc. mg</th>
<th>Total turnover rate mg/day</th>
<th>Turnover rate as percent plasma pool/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.87±1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18.66±3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>24.58±1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>24.06±1.1</td>
<td>4.23</td>
<td>17.61</td>
</tr>
<tr>
<td>8</td>
<td>21.79±2.2</td>
<td>3.84</td>
<td>17.62</td>
</tr>
<tr>
<td>10</td>
<td>20.63±1.9</td>
<td>3.63</td>
<td>17.59</td>
</tr>
<tr>
<td>13</td>
<td>31.76±2.8</td>
<td>5.59</td>
<td>17.60</td>
</tr>
<tr>
<td>17</td>
<td>34.75±2.9</td>
<td>6.12</td>
<td>17.61</td>
</tr>
<tr>
<td>21</td>
<td>62.67±4.4</td>
<td>11.03</td>
<td>17.60</td>
</tr>
<tr>
<td>Mean ± σ</td>
<td></td>
<td></td>
<td>17.61±.004</td>
</tr>
</tbody>
</table>
difference between total plasma pool and the calculated maternal
contribution should be de novo synthesized IgG.
DISCUSSION

Metabolism and passive transfer of immunoglobulins in the turkey hen

It was assumed throughout these experiments that purification and labeling procedures did not alter the metabolic behavior of immunoglobulins. Evidence presented in Fig. 1 indicated that injected labeled Ig's were chromatographically indistinguishable from corresponding unlabeled Ig's of serum and, during the first experiment, in yolks. It was further assumed in experiment 1 through 4 that pool size, exchange rates, and rate of synthesis and catabolism remained constant.

Purified turkey serum IgG (.06M P04 fraction from DEAE) was used in experiments 1 and 2. We have found no evidence that this preparation contained distinctive subclasses of IgG, based upon the results of I.D., I.E.P., and ultracentrifugation studies (Saif and Dohms, 1976; Dohms, unpublished results). Furthermore, theoretical expected concentrations of IgG calculated from accumulated isotope levels in egg yolks agree with the results of RID assays of yolk IgG levels in the two birds examined (calculated as follows for each bird: percent of injected dose accumulated in ova during the first 19 days X average eggs layed/day X total mg IgG lost/day). Thus, the .06M DEAE fraction appears to account for most if not all the IgG found in ovo. This does not preclude the possibility of more than one
metabolically distinct population existing within the serum, egg yolk, or the .06M IgG fraction.

The passive transfer of IgG from turkey hen serum into the egg is part of the overall metabolism of the IgG class. Injected labeled IgG was distributed into two pools with different half-lives. The rapidly eliminated component, about 60% of the injected dose, is believed to represent equilibration between intravascular and extravascular pools, with the remaining 40% representing IgG decay after equilibration. It is possible that initial equilibration pool contains some IgG with a faster turnover rate. The two-pool model could also be interpreted to represent a precursor-product relationship, but this can be ruled out since chromatographic data indicated that the radioactivity peak was associated with IgG throughout the experiment. The final linear decay slope with an intravascular space of 40% is about 8 - 10% lower than the intravascular space reported from human IgG (Waldman et al., 1971) and in other mammals (Sabiston and Ste. Rose, 1976; Sasaki et al., 1976).

The mean total IgG turnover rate of .062±.01 gms/kg/day can be expressed as 11.92±.01% loss of the total IgG plasma pool/day. It may also be expressed as the percent of the estimated total body pool IgG content (1.28 g IgG per kg assuming 40% of the body pool is intravascularly distributed) so that approximately 4.8% of the total body IgG is turned over per day. By assigning total IgG content of turkey ova (83.97 mg which is the mean of 3 experimental birds tested by RID assay) a loss of 2.34% of the plasma pool is lost/day if the laying rate is 100% (one egg laid per day). Assuming a more realistic
60\% \text{ rate of lay results in a calculated loss of 1.4\% of the plasma pool per day. Recalculating these losses for estimated whole body level of Ig results in estimates of 95\% loss at 100\% rate of lay and 57\% at 60\% rate of lay. Thus, transfer of Ig to egg ova represents a small fraction of the overall daily irreversible loss of Ig. This situation is in contrast to the large loss of Ig}.

It is not possible to make comprehensive comparisons of Ig metabolism in other avian species since studies have been conducted only in chickens, and total turnover studies were often not conducted. The IgG (presumably Ig) half-lives of 1.5, 3, 2.5, and 4.0 days were reported in laying White Leghorns, newly hatched chicks, 16- to 16-week-old male, and non-laying female White Leghorns, respectively (Patterson et al., 1962b; Phillips, 1966; Westerman and Olson, 1964). In the only study using purified Ig-G labeled Igs, Leslie and Clem (1970) reported a 4.1 day half-life for Igs (chicken Ig-G), a serum concentration of 5.29+1.3 mg/ml, fractional turnover of 16.9\%, and a total body turnover (or synthetic rate if at steady state) of 56 mg/kg/day in adult roosters.
and turkeys (Nestor et al., 1970). The middle sized (those ova that have the most rapid accumulation rate of macromolecular constituents) ova would be exposed to the highest initial levels of labeled IgG in the blood over the longest period of time. Fig. 2 (experiment 2) supports this idea since middle sized ova (approximately 15 - 70% developed) had the highest accumulated levels of labeled IgG.

The parallel decay of radioactivity in blood and egg yolk indicated that within individual hens IgG transfer proceeds at a fairly constant rate. This is supported by the results of RID assays on representative egg yolks of three hens that showed a constant overall trend in IgG content in ova. The random variation observed in these assays was attributed to the variations in assay and percent recovery which were 4.0 and 11.1%, respectively. Heller (1975) has shown variation in yolk antibody levels to bacterial antigens (2 ME resistant, presumably IgG) in sequentially laid chicken eggs. This may have reflected the variability of antibody recovery from yolks or that changes in antibody levels in the sera of hens were undetected by serologic tests. The observed parallelism noted in this experiment is affected by reproductive performance, since laying pauses due to irregular ovoposition tend to flatten the decay curve. This is especially noticeable in hen 2, Fig. 2. Therefore, in other strains of turkeys exhibiting poorer reproductive performance, the phenomenon of parallel yolk and blood decay may not be apparent. Previous work with White Leghorns showed a rapid fall of $^{131}$I labeled gammaglobulin in sequentially laid eggs which was attributed to the observed fast half-life of 1.6 days. However, a graphic comparison with serum decay
rates or a half-life determination in sequentially laid yolks was not made in this study (Patterson et al., 1962a).

Although overall passage of IgG appears to remain constant, the transfer rate of IgG in developing ova occurs in a variable but predictable manner in the ovary (Fig. 3). In these studies, as well as previous studies in chickens, the middle sized ova had the greatest rate of accumulation per cm² surface area (Patterson et al., 1962a,b). The only observed differences between the turkey and chicken was the greater number of middle sized ova in turkeys exhibiting a high level of transfer of IgG, a reflection of the greater number of ova in rapid development. The time of rapid accumulation and the pattern of accumulation of IgG correlates closely with the transport of other yolk constituents during rapid growth, and with overall growth rate of the chicken ova (MacKenzie and Martin, 1961; Warren and Conrad, 1939). Thus, uptake of IgG is one part of the transfer of macromolecular yolk constituents.

Previous work comparing antibody titers in hen's serum with the corresponding egg aqueous yolk fractions (corrected for percent recovery and assumed 50% solids/ova) showed that the titers of the yolk fractions exceeded or were equal to serum titers (Patterson et al., 1962b). Other workers using various serologic techniques of varying sensitivity have reported yolk levels to be from 13 to 100% of the serum titers (Brambell, 1970). Using labeled IgG, we have found that the aqueous fraction of turkey yolk averages 79.15±2.78% of the plasma IgG concentration (range 73.6±31.7%) assuming that yolk is 50% solids.
Differences in the IgG content of egg yolks were observed among the small number of hens examined using the RID assay. It is apparent from experiments 1 and 2 that several factors may affect the levels of IgG passed into turkey eggs. In chickens it was suggested that hens laying the largest ova have the most immunoglobulin (Kramer, 1973). In experiment 2, Fig. 2, two hens each had about the same rate of IgG transfer per cm$^2$ surface area. Since the follicular epithelial cells surround the ova, this rate of transfer reflects the transfer per cm$^2$ of epithelium. Therefore, a hen laying larger ova would be expected to have more surface area and therefore more total IgG. However, quantitative assays in yolk from three different hens (experiment 1), indicated that hen 2 had a statistically significantly higher level of total IgG after correction for weight differences (egg weight, size, and surface area are positively correlated). Thus, it is apparent that some other mechanism may also determine egg yolk IgG levels. Interestingly, this same hen had the highest IgG concentration in plasma, and the fastest turnover rate and half-life. The high level of IgG in the yolk may reflect the inverse relationship of concentration and turnover rate observed in mammals (Brambell, 1973). Perhaps the greater concentration of IgG allows a greater saturation of receptor sites necessary for transfer of IgG across the follicular epithelial cells. Another possible explanation is that follicular epithelial cells in some hens may possess varying abilities to pass IgG into ova, a situation not readily observed in experiment 2 where only two birds were examined.
Some investigators have been unable to detect IgG in the egg whites of unincubated chicken eggs (Kramer and Cho, 1970; Rose and Orleans, 1974), whereas it was detected by others in turkeys (Saif and Dohms, 1976) and chickens (Yamamoto et al., 1975) using I.D. assays after, in some cases, extensive concentrations of egg white. We have found IgG in unincubated turkey egg whites without concentration in 100% of the eggs examined using I.D. assay but could not quantitate the IgG in unconcentrated whites using RID assay with a threshold of sensitivity of 20 µg/ml. Since the I.D. sensitivity is about 10 µg/ml in our system, we estimate that only about 350 to 550 µg IgG would be present in the white of an egg, or about .4 to .6% of the yolk IgG level per egg. At least some of this is derived from serum transudation since about 50% of the egg white radioactivity at peak levels was precipitated with 10% TCA. It is possible that some of the IgG could be derived by local synthesis in the reproductive tract. We suggest that this low level of IgG in egg white is an insignificant contribution to the overall phenomenon of passive IgG transfer in the turkey.

Data on the IgM radioactivity time curve for hen 7 appears to indicate that either very fast or no equilibration took place between the intravascular and extravascular pools. This may be similar to situations in rabbits (Taliaferro and Talmage, 1956) and humans (Cohen and Freeman, 1960) where IgM is found to be distributed mostly in the intravascular space. However, 24 to 48 hrs time was required before experimental decay occurred in roosters injected with homologous 16.75 IgM (Leslie and Clem, 1970). Careful measurements in rabbits
indicated that 71.0±0.04% was distributed into the intravascular space (Sabiston and Ste. Rose, 1976). The IgM half-life of 2.69 and 2.74 days (fractions turnover 25.8 and 25.3%) is longer than the average 1.7 day half-life (fractional turnover 40.76%) of IgM in roosters (Leslie and Clem, 1970). By quantitating IgM in serum, Leslie and Clem (1970) were able to calculate total turnover (synthetic rate if synthesis = catabolism) of 16 mg/kg/day; a value not reported in this study since serum IgM levels were not determined. The very low level of protein precipitated radioactivity in yolk plasma indicates that the amount of IgM antibody is extremely low and of questionable significance. Although detected in chickens' ova using I.D. assay, it required tenfold concentration (Yamamoto et al., 1975). Attempts to detect this class of Ig in turkey egg yolks using I.D. assay with tenfold concentration with ammonium sulfate precipitation of aqueous yolk were unsuccessful but may reflect the insensitivity of the our system.

Turkey Biliary IgA chromatographs on G-200 at the void volume in an identical position as turkey IgM and high molecular weight serum IgA. A lower molecular weight serum form has been identified in turkeys and chickens but neither high nor low molecular weight serum forms were purified from turkeys (Dohms et al., 1976; Leslie and Martin, 1973; Higgins, 1976). Biliary IgA also shows complete identity with high molecular weight serum IgA on I.D. developed with rabbit anti-biliary IgA monospecific for the alpha chain. Thus, chromatographically and antigenically biliary IgA is comparable to high molecular weight serum IgA (Dohms et al., 1976). The IgA specific
radioactivity time curve must be cautiously interpreted since the possibility exists that more subtle metabolic differences exist between these molecular forms of IgA. In contrast to IgM of the same approximate molecular weight, IgA was distributed into two pools with 95% with a fast half-life of three hours and a residual component of about 5% with a slower half-life and turnover rate. The following possibilities exist to explain the data: 1) the presence of a large extravascular pool for biliary and high molecular weight serum IgA is expected and the distribution data reflect the true equilibration of this immunoglobulin; 2) the distribution and half-life is true for only biliary IgA and not high molecular weight serum IgA; 3) mixed forms are found in bile (and/or serum) and have different half-lives, 95% with 3 hr and 5% with a half-life about 1.83 days; 4) extensive damage to protein occurred resulting in the rapid removal of a larger part of the injected dose. The last seem unlikely since heavily damaged proteins are reportedly eliminated in a matter of minutes (Freeman, 1959). Like IgM, small amounts of TCA precipitable radioactivity was found in egg yolks from IgA injected hens agreeing with results of one previous report indicating IgA identification in chicken yolks after I.D. testing of yolk material concentrated ten times (Yamamoto et al., 1975). Yolk fractions concentrated tenfold were tested using I.D. with rabbit anti-α sera and no precipitin line was detectable. Therefore, like IgM, IgA appears not to substantially contribute to passive Ig transfer in turkey yolks.

The presence of large amounts of free iodine in egg yolks of IgM and IgA injected hens requires comment since no free iodine was
detected using G-200 chromatography in yolks of IgG $^{125}$I injected hens. It is possible that the more rapid catabolic rates of IgM (approximately 2 X IgG) and IgA (approximately 4 X IgG) generated larger amounts of free iodine over a shorter time span that allowed entry into the developing ova, following rapid clearance from the blood (Fig. 1). This idea is supported by the fact that a larger percentage of the injected dose appears as free iodine in yolks of IgA injected birds (higher fractional turnover rate) than the yolks of IgM birds.

In summary, IgG is the predominant Ig involved in the passive maternal transfer process in the turkey hen. Its contributions to egg yolks of between 73 to 104 mg/egg represents less than one percent of the total estimated body pool of IgG. In individual hens, it appears that IgG passes onto eggs at a constant rate. This was most easily observed in birds with high egg production and showing regular ovoposition. Metabolic data indicated that IgG had the longest half-life and turnover rate of the three Ig classes examined with respective half-lives for IgG, IgM, and IgA of 5.9, 2.9 and 1.97 days.

When designing a disease control program involving transfer of maternal immunity to poults, it is important to reemphasize that hens transfer only IgG into eggs in significant amounts. Thus, it is important to find out the Ig class and amount of an antibody that would protect hatching birds against a specific infection. In addition, in evaluating the serological status of breeder flocks it is important to consider the antibody class responsible for the titer observed in the assay. For instance, a high titer of predominantly
IgM antibody is not helpful in maternal antibody since IgM is not passed in significant amounts. Another important point revealed by this study is that different hens can have significantly different levels of IgG passed into eggs which are independent of egg ova size or weight. If this exists in commercial strains it is conceivable that such variability may allow selection of high or low levels of IgG in egg yolks. Combined with selection for high IgG-antibody responders in hens to particular pathogens may allow the development of strains of birds that have extended periods of maternal immunity. The variability of IgG levels between birds is of more immediate importance in considering that pouls may possess a wide range of IgG levels within a hatch resulting in variable levels of maternal IgG and therefore variable times under the influence of maternal immunity.

The metabolism and levels of IgG in the newly hatched turkey poul

During purification procedures our observations were that the .06M phosphate buffered DEAE fraction contained the major amount of yolk IgG. There was no evidence of more than one IgG subclass as indicated by IEP. Gel filtration and I.D. studies indicated that the IgG was not affected by the purification procedures. The trace iodinated IgG was unaltered structurally when assessed by G-200 chromatography (Fig. 5).

The intrabdominal route of injection was chosen because of the difficulty of injecting young birds intravenously via the wing veins or the heart. Nonetheless, it is possible that some of the injected dose was inadvertently placed in the yolk sac. The intravascular and
extravascular equilibration of 3 to 6 days is similar to values obtained for intravenously injected turkey hens (data from exp. 1).

The great increase in plasma volume in domestic birds corresponds to the increase in body weight which is roughly doubling every seven days. This has been confirmed experimentally in chickens (Medway and Kare, 1959). The half-life of IgG in the total plasma pool of 4.08+.33 days is longer than the 2.88+.09 day half-life measured in a fixed one milliliter plasma volume since it adjusts for the dilutional effect of expanding plasma volume. The difference in half-life determinations between the two methods points out the danger of attempting to use serology for a specific antigen in sera collected at different intervals after hatch to estimate half-life. Such estimates ignore the increasing plasma pool compartment during growth.

When plasma volumes were calculated the assumption was stated that blood volume is 10% of the total body weight. This is not based upon experimental evidence in turkeys but was an assigned value. If another value is used for blood volumes, the plasma pool half-life would remain unaffected. If the blood volume as percent body weight had changed during the study, the standard error of the regression line for radioactive decay would increase. Medway and Kane (1959) found decreasing blood volumes as percent body weight in growing White Leghorn chickens. A 12.6+.9% blood volume during the first week dropped to 10.4+.9% blood volume the second week, and 0.7+.5% the third week of life. If a similar drop occurred in turkeys this would affect the total plasma decay curves very little since data was calculated
from 6 through 21 days PI corresponding to the 10.4±.5% to 9.7±.5% values at these ages in chickens.

In young chickens a three day half-life of iodine$^{131}$ labeled gammaglobulin was reported during the first week post-hatch. The same authors measured the gammaglobulin half-life in adult hens and reported a 1.5 day half-life (Patterson et al., 1962a). We report a half-life value of 4.08±.33 days in turkey pouls measured from days 7 through 22 after hatching which is shorter than the 5.9±.41 day value observed for the adult turkey hen (exp. 1). Presented another way, a calculated total turnover rate of 11.9% of the plasma pool/day in adult hens is less than the 17.6% total turnover rate of plasma pool per day for the pouls (Table 9). This is consistent with the idea that metabolic rate is faster in smaller than larger animals of the same or similar type of species (Prosser and Brown, 1950).

The data presented in Fig. 7 showing the percent decay of the injected dose remaining in the plasma pool is based upon the assumption that the half-life of IgG on days 1 through 5 PI is the same as from day 6 through 21 post-injection. The decay of the injected dose at $T_1$ may be compared to maternal immunoglobulin. It is like maternal IgG in that it is a fixed concentration of egg yolk IgG passively transferred. It is unlike maternal IgG in that it is administered at a particular point during the transfer process of IgG from yolk to the poult and it represents a small fraction of total IgG pool present in poult's at the time of injection.

In chickens nonagglutinating antibody to *Salmonella* was detected in embryonic serum from 11 days embryonation onward until hatching at
21 days (Buxton, 1952). Leslie and Martin (1973) quantitated levels of IgG at 15 days and found them to be 2 to 4% of hatching serum concentrations. Thus most of the IgG at hatching is passaged into the embryonic circulation in the last 5 to 6 days before hatching. After hatching the yolk sac continues to contribute nutritional factors as well as maternal IgG to the neonate for a short period after hatching (Brierly and Hemmings, 1956). In the turkey we found by a time corresponding to 1 day PI only 36% of the yolk sac wet weight remained of an original average 6.6 gram weight. Therefore the injected dose and its decay are similar to but not identical with maternal IgG because it is passively transferred (injected) in a narrower time range than naturally transferred egg yolk IgG.

Quantitation of plasma IgG using the RID assay provided IgG concentration per milliliter as indicated in Fig. 8. These values at hatching and at 6 days PI are similar to values obtained from chickens (Leslie and Martin, 1973; Martin and Leslie, 1973; Van Meter et al., 1969). The data for plasma pool IgG levels which are based on the blood volume assumptions discussed above are presented in Fig. 8. Combining information on percent decay of the injected dose at T1 with the total plasma pool data allowed construction of a theoretical model for maternal IgG decay in the plasma pool (Fig. 8). The limitations of the model include: 1) that is assumes that all maternal antibody is present in the pool by T1, and 2) that this IgG is exclusively of maternal origin. Since there is no statistically significant rise in levels at day 0 through day 6 it may well represent most of the maternal IgG pool. The contribution of the remaining IgG in the yolk
sac can only be estimated on the basis of a mean value of IgG concentration in unincubated eggs and the data on yolk sac absorption. Assuming 50% distribution of this material into the intravascular space, we estimate that as much as 6 to 8 mg of IgG can be added to the plasma pool from day 1 through 2 PI, thereafter contribution from the yolk sac rapidly decreases in significance. Even after these upward estimates, by day 3 a marked difference is observed between the total IgG pool and estimated maternal contribution to the pool. This is believed to be actively synthesized IgG. Synthesis of IgG may have begun earlier but is masked by the large amount of maternal IgG.

In chickens the earliest reported detectable IgG synthesis is eleven days post-hatching as measured by the use of allotypic markers (Khahab and Craig, 1970). Leslie (1975) discussed unpublished observations of de novo synthesis of IgG at 3 to 4 days post-hatch in a group of chicks raised from a hen with severe IgG hypogammaglobulinemia. The presence of actively synthesized IgG early after hatching is not surprising since the immunocompetence of the chicken embryo is clearly established (Solomon, 1971). Using a sensitive technique of Reticuloendothelial clearance to measure antibody production to an erythrocyte antigen, Solomon (1966) detected opsonizing antibody at 3 days post-hatch after primary sensitization of embryos at 14 days of incubation although the Ig class was not designated.

In conclusion, the data provided in this study indicate that during the first week of life IgG synthesis increased as the yolk sac IgG contribution decreased with the net result of stationary or slightly elevated levels of IgG in the vascular and extravascular body
compartments. During this time elimination of maternal IgG by
catabolism was replaced by the influx of actively synthesized IgG.
By the second week, synthesis had increased and expanded the total body
pool. Irreversible loss was 17.61% of the plasma pool per day. At
this time the maternal yolk sac IgG addition was insignificant if
present at all and approximately 18% of the total intravascular pool
was of maternal origin. Increased IgG synthesis continued into the
third week with a continuing decline of maternal IgG. Total
irreversible loss of IgG continued to remain at 17.61% of the plasma
pool/day.
SUMMARY

Studies were initiated to determine the metabolism and the basic mechanisms by which turkey immunoglobulins G, M, and A are passively transferred from the turkey hen into the egg. The half-life of $^{125}$I-labeled IgG was calculated to be 5.91±.81 days and the time of maximal passage of radioactivity from hen into eggs was 6.51±1.3 days. The following IgG determinations were made: plasma level (8.54±1.2 mg/ml), total plasma pool (.518+.07 gm/kg), total turnover rate (.062+.01 gm/kg/day), or 11.92±1.83% of the plasma pool per day.

The overall passage of IgG into sequentially laid eggs of individual hens was at a constant rate over an observation period of 46 days, and loss to egg yolks accounts for less than one percent of the total daily irreversible loss of IgG from the hen's body. The IgG levels in egg yolks assayed in three hens ranged from 72.1±6.7 to 104.5±6.9 mg per yolk with significant differences observed between birds not accounted for by differences in egg weights. IgG was detectable in egg whites but at a low level. The biological significance of egg white IgG is questionable.

Iodine$^{125}$ labeled IgM had a half-life of 2.69 and 2.74 days with fractions turnovers of 25.76 and 25.29% of the plasma pool per day in two hens examined. IgM-associated radioactivity in egg material was considered insignificant in overall immunoglobulin transfer into eggs.
Iodine$^{125}$ labeled IgA (biliary) had a half-life of 1.92 and 1.68 days with fractional turnovers of 36.09 and 41.25% of the plasma pool per day. Like IgM, IgA-associated radioactivity in egg material was considered insignificant in overall immunoglobulin passage into eggs.

Studies were also conducted to determine the persistence of maternal IgG in the hatched turkey poult using Iodine$^{125}$ labeled egg yolk IgG injected near hatching. Determinations were made of the half-life of IgG in the total plasma pool. Results indicated a half-life for IgG of 4.08±.33 days. Elimination of the injected IgG, which was comparable to maternal IgG, was plotted as the percent of the injected dose remaining in the plasma with 30.53, 10.31 and 3.48 percent of the injected dose present at 7, 14, and 21 days after injection. IgG levels in young poults were examined by radial immunodiffusion assay and it was estimated that de novo IgG synthesis was possibly observed as early as three days after hatching.
BIBLIOGRAPHY


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Cripps, A. W. and A. K. Lascelles, 1974. The biological "half-lives" of IgG$_1$ and IgG$_2$ in young milk-fed lambs and in non-pregnant colostrum-forming sheep. Aebak. 52: 717-719.


