Chicken Immunoglobulins: Biological Half-lives and Normal Adult Serum Concentrations of IgM and IgY[1] (34758)

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Chickens have two classes of serum immunoglobulins, which differ from one another antigenically and in several physicochemical properties (1, 2). The 16.7S immunoglobulin resembles human IgM whereas the 7.1S immunoglobulin does not readily align itself physicochemically with any of the known human immunoglobulin classes. In fact, Mehta and Tomasi (personal communication), employing complement fixation procedures, have shown that while human IgM cross reacts with chicken IgM, human IgG does not cross react with the chicken 7S immunoglobulin. This lack of a close correlation between the chicken 7.1S immunoglobulin and any of the described human immunoglobulins has led us to give it the interum designation of IgY (2, 3). However, in spite of an apparent lack of structural homology, chicken IgY may be biologically analogous to mammalian IgG. For this reason, it was deemed important to determine the biologic properties of the two classes of chicken immunoglobulins.

The present investigation measures the half-lives and adult serum levels of the two classes of chicken immunoglobulins and compares these biological properties with those of human immunoglobulins.

Materials and Methods. Purification of immunoglobulins. Chicken IgM (1, 4) and IgY (2) were prepared as described previously.

Quantification of immunoglobulins. Antisera were prepared in rabbits by injecting purified heavy (H) chains in complete Freund adjuvant (2).

Radial immunodiffusion was carried out essentially as described by others (5, 6). The sera were obtained from 6 to 8-month-old birds. The concentrations of the control IgM and IgY proteins were determined using an extinction coefficient ($E^{\%}_{280\ m\mu,\ 1\ cm}$) of 12.7 and 13.2, respectively (2). Diffusion times were 18–24 hr for IgY and 48 hr for IgM. Each of the standards and unknown were tested in duplicate and the precipitin ring diameters were averaged.

Radioiodination of IgM and IgY. The chloramine T method as described by McConahey and Dixon (7) was used.

Half-life ($T_{1/2}$) determinations. One ml, containing approximately 1 mg of the ^{125}I-labeled immunoglobulin, was injected into the jugular vein. Blood samples were taken 2 and 15 min; 4 hr; and 1, 2, 3, 4, 5, 6, and 7 days after injection. The radioactivity in measured aliquots of sera was counted in a well-type NaI scintillation detector at the conclusion of the experiment.

Results. Quantitative radial immunodiffusion of the purified IgM and IgY standards demonstrated a linear relationship between the logarithm of protein concentration and the precipitin ring diameter over the concentration range employed (0.17 – 5.6 mg/ml of IgY and 0.09–3.1 mg/ml of IgM). In order to circumvent fluctuations in ring size which may result from assay to assay, standards were run on the same plate as the unknown sera. Table I shows that the average serum

TABLE I. IgM and IgY Immunoglobin Levels in Adult Chicken Serum.

Immunoglobin	Range	Av + SD	IgY:IgM ratio
IgM	0.5–0.93 (9)[a]	0.71 ± 0.18	7.5:1
IgY	4.1–7.3 (6)[a]	5.29 ± 1.35	

[a] Number of birds.

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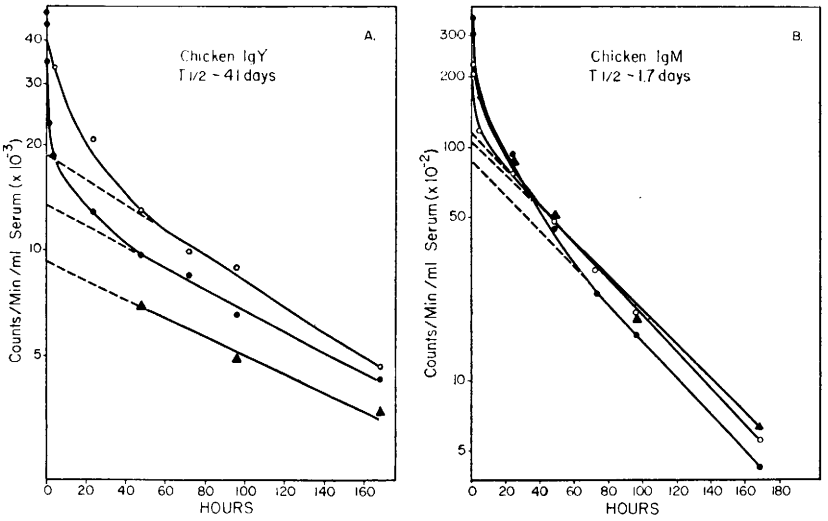

FIG. 1A. Serum elimination curves of ^{125}I-labeled chicken IgY. (B) Serum elimination curves of ^{125}I-labeled chicken IgM.

concentrations of IgM and IgY were 0.71 mg/ml and 5.29 mg/ml, respectively. The serum IgY and IgM ratio was approximately 7.5:1. It is well recognized that purified chicken IgM contains variable amounts of high molecular weight aggregates (2, 4, 8) which would tend to make the radial immunodiffusion values for IgM slightly lower than they really are.

Homologous radioiodinated chicken IgY and IgM were injected intravenously into adult roosters and blood samples were removed at various times, allowed to clot, and the sera were analyzed for radioactivity. The serum radioactivity profiles obtained with passively administered IgY are shown in Fig. 1A. Two out of the three animals showed a rapid clearance of radioactivity followed by a slower first-order elimination. Several early bleedings from the third animal were lost but the remaining samples allowed part of the elimination curve to be drawn. The 7S IgY appeared to be completely equilibrated within 48 hr after administration. This period of rapid clearance is most probably due to a combination of intravascular and extravascular equilibration and the removal of aggregated or denatured immunoglobulins. When the serum radioactivity profile approximates a first-order decay, it is possible to calculate the biological half-life. These calculations are shown in Table II and show that IgY has a half-life of approximately 4.1 days.

Figure 1B depicts the radioactivity profiles of serum obtained from birds passively administered ^{125}I-labeled chicken IgM. As was shown for the passively administered IgY, there was a rapid elimination of radioactivity for 24 to 48 hr followed by approximate first-order elimination. The half-life obtained in each of the three animals is given in Table II. The average half-life for IgM was approximately 1.7 days.

Discussion. The main point to be covered in this discussion is that, based upon biologic properties such as half-lives and serum concentrations, chicken IgY and IgM may be analogous to IgG and IgM of higher animals.

Earlier studies on the metabolism of chicken gamma globulin (presumably 7S immunoglobulin) showed half-lives of about 1.5

TABLE II. Biological Half-lives of Chicken IgM and IgY.

Immunoglobulin	Animal	Days	Av (days)
7S IgY	1	4.0	
	2	4.8	~4.1
	3	3.5	
16.7S IgM	4	1.6	
	5	1.8	~1.7
	6	1.7	

days in adult hens and 3 days in newly hatched chicks (9), about 4 days in 90- to 110-day-old germfree and conventional chickens (10) and about 2.5 days in 4-week-old birds (11). Whether or not the gamma globulin preparations used in the above studies contained proteins other than IgY is not clear; thus it is difficult to compare the half-life of 4.1 days obtained herein with the previous studies. It is also quite difficult to make meaningful comparisons between mammals and chickens in this respect due to highly variable values reported for the former. For example, values of 20 days (12, 13) and 12 days (14) have been reported for the half-life of human IgG whereas those of mouse and rabbit IgG are ~4 days (15) and ~5.5 days (16). Differences in half-lives of human IgG myeloma proteins associated with subclass specificity have also been reported (14). The half-lives of human IgA (6 days (17)) and IgD (2.8 days (18)) are considerably lower than for IgG.

The approximately 2-day half-life obtained for chicken IgM is somewhat lower than the 4–5-day half-life generally associated with human IgM (19–21), although values as low as 2–3 days have been reported for the latter protein (12). On the other hand mouse IgM has a half-life of only 0.5 days (15). Previous workers have shown that the level of 7S immunoglobulin in chicken serum ranged from 7.3 mg/ml in hyperimmunized birds to 5.9 mg/ml in normal 12- to 13-week-old birds (22) to 2.7 mg/ml in 44-day-old birds (23). The results reported here ranged from 4.1 to 7.3 mg/ml with an average of ~ 5.3 mg/ml. This value is in close agreement with the previous values for adult chickens but is considerably lower than the ~12 mg/ml of IgG found in normal human sera (19). The adult serum level of chicken IgM was determined to be ~0.71 mg/ml. This is quite similar to the IgM level in adult human serum (19).

The blood volume of a chicken is approximately 9% of the body weight or 90 ml/kg (24). By knowing the serum concentration of IgY (5.3 mg/ml) and the half-life (4.1 days) a synthetic rate of ~58 mg/kg/day can be calculated for serum IgY. Similar calculations show that chicken IgM has a synthetic rate of ~16 mg/kg/day. Thus in chickens a total of ~74 mg of serum immunoglobulin/kg/day are synthesized which is very similar to the ~80 mg/kg/day calculated for man (19). However, the chicken has a synthetic rate for IgM which is about 3 times that of human IgM.

In conclusion, it can be stated that chicken IgY is present in a higher serum concentration and has a longer half-life than does chicken IgM. Since similar relationship is seen with human IgG and IgM, it is tempting to suggest that chicken IgY and IgM are biologicaly analogous to human IgG and IgM, respectively. The well-established fact that most of the serum antibody in hyperimmunized chickens is of the 7S-type (IgY) can also be interpreted as supporting the suggestion that chicken IgY and human IgG are biologically analogous. However, it should be emphasized that although chicken IgM appears structurally homologous to human IgM, chicken IgY can not be readily aligned with any described human immunoglobulin class.

Summary. Chicken IgY, the major serum immunoglobulin, was shown to have a biological half-life of ~4.1 days, a serum concentration of ~5.3 mg/ml, and a synthetic rate of mg/kg/day. Serum IgM had a half-life of ~1.7 days, a serum concentration of ~0.71 mg/ml, and a synthetic rate of 16 mg/kg/day.

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