The Metabolism of Serum Proteins in the Hen and Chick and Secretion of Serum Proteins by the Ovary of the Hen

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ABSTRACT In the chicken, serum gamma globulin (CGG) is preferentially transferred by the follicular epithelium of the ovary to the developing ova. The concentration of gamma globulin in the yolk of the unfertilized egg is many times the concentration of chicken serum albumin (CSA). This transfer occurs largely during the 4 to 5 days preceding ovulation when the growth of the ovum is most rapid. Thus, in the chicken, the follicular epithelium of the ovary serves the same purpose in the passive immunization of offspring as does the acinar epithelium of the udder in ungulates and the extraembryonic membranes in rabbits and man.

The amount of gamma globulin synthesis by the chick is low during the first 2 weeks of life and is associated with low levels of serum gamma globulin. By the end of the 1st month of life, the level of serum gamma globulin increases, presumably reflecting an increased rate of synthesis.

In the adult hens the half-life of I^{131}-labeled CSA is 66 hours and that of I^{131}-labeled CGG, 35 hours, while in the newly hatched chick for I^{131}-labeled CSA it is 42 hours and for I^{131}-labeled CGG, 72 hours. Thus, this species shows a gamma globulin sparing in the first days of life, as do most mammalian species.

The secretion of serum proteins by the chicken ovary into the developing ova has several interesting features and consequences. It provides a useful model for studying transport of serum proteins and specific antibody across an epithelial membrane in different stages of development, since various stages of the developing ova, each surrounded by the ovarian follicular epithelium in a corresponding stage of development, are present simultaneously in a single laying hen. The passage of gamma globulin from the

hen to the egg yolk is the means of maternal transfer of antibody to the chick. The loss of serum proteins from the hen to the yolk is of interest in the study of metabolism of serum proteins in this species. The transovarian passage of maternal antibody in birds provides an interesting comparison with the various means of maternal to fetal transfer of antibody in mammalian species. The present studies were designed to observe the passage of serum gamma globulin and serum albumin to the yolk along with related studies on the metabolism of serum proteins and antibody in the hen and developing chick.

METHODS AND MATERIALS

I^{131}-Labeled Proteins Gamma globulin was prepared by fractionation of pooled normal White Leghorn chicken sera with ammonium sulfate as described by Sternberger and Peterman (1). Serum albumin was prepared using a modification of the method of Schwert (2). In this procedure, globulins were precipitated from the serum by 50 per cent saturation with ammonium sulfate. After dialyzing the supernate free of ammonium sulfate, the remaining protein was precipitated by the addition of trichloroacetic acid at a final concentration of 5 per cent. The precipitate which formed was packed by centrifugation and then brought into solution with absolute alcohol. Under these conditions, only the native albumin is soluble in absolute alcohol. The alcohol was removed by dialysis against 0.15 M NaCl. The entire fractionation was carried out at 0–1°C. Paper electrophoretic analyses of the serum albumin and serum gamma globulin fractions with a Spinco model R paper strip apparatus are illustrated in Fig. 5B and C. Protein nitrogen content of the fractions was determined by the Markham modification of the micro-Kjeldahl technique (3). The albumin solution contained 11.25 mg protein/ml, and the gamma globulin solution contained 4.48 mg protein/ml. The chicken serum albumin (CSA) and the chicken serum gamma globulin (CGG) were trace-labeled with I^{131} (I*) by the method previously described (4).

Determination of Half-Lives of Serum Proteins in Hens and Chicks Adult laying hens were injected intravenously with either I* CSA or I* CGG and blood was drawn at daily intervals. Determinations of serum protein–bound I* were made in a well-type Geiger gamma counter, and the half-lives of the serum proteins in the circulation were determined graphically. One day old chicks were injected intravenously with either the I* CSA or the I* CGG and the total radioactivity in the young birds was determined every 2 days by counting the chicks in a large well-type Geiger counter. Representative chicks were homogenized in a Waring blendor at 2 day intervals, and the protein-bound I* determined. At least 95 per cent of I* in the chick homogenates was protein-bound. The half-lives of the I* CSA and I* CGG in the chicks were determined graphically.

Blood Volumes of Chickens Blood volumes were calculated from the dilution of a known amount of the I* CSA or I* CGG injected intravenously in the adult hens. Bleedings were done 10 minutes after injection of the labeled protein.

Determination of I CSA and I* CGG in Ovarian Ova and in Sequentially Laid Eggs* Adult laying hens were injected intravenously with either I* CSA or I* CGG. Some of these hens were sacrificed 24 hours after injection. Their ova were removed from the ovary and the protein-bound I* present in the yolks was determined. Other hens were maintained and continued to lay eggs after injection and protein-bound I* was determined in the yolks of successively laid eggs. Aliquots of yolk material obtained either from the ovarian ova or from the eggs laid after injection were extracted with ether, ethylene chloride, and 0.15 M NaCl, using the method described by Schmittle (5), with the exception that all yolk material was first removed and thoroughly mixed prior to removal of the aliquot for extraction. The protein-bound I* of the saline extract was determined by precipitating with trichloroacetic acid and counting in a well-type Geiger counter.

Measurement of Antibody in Sequentially Laid Eggs Laying hens were immunized with a single intravenous injection of 50 mg/kg body weight of bovine serum albumin (BSA), Armour and Co., lot S68108. The yolks of eggs and serum samples obtained after immunization were analyzed for anti-BSA antibody levels by the method of Talmage and Maurer (6). Yolk extracts for antibody determinations were obtained as described above and were freed of organic solvents by bubbling nitrogen through them. Sufficient NaCl was added to sera and yolk extracts to give a final concentration of 8 per cent prior to antibody determination (7).

Autoradiography Autoradiographs of egg yolks containing I* CGG were prepared by slicing 2 to 5 mm cross-sections of egg yolks which had been fixed in 10 per cent formalin. The sections were dried and placed in a contact with Eastman Kokak contrast lantern plates. Several plates were prepared and developed after varying periods of time ranging from 2 days to 2 weeks to establish the appropriate exposure time. The lantern slides were developed in Eastman Kodak dektol 1:1.

Serum Protein Patterns of Developing Chickens Embryos and chicks were bled at various intervals beginning 2 days before hatching, and the sera analyzed by paper electrophoresis using a Spinco model R paper strip apparatus. The total protein nitrogen content of the sera was also measured.

All hens and chicks used were white Leghorns. All birds injected with I*-labeled proteins had 1:10,000 KI in their drinking water prior to and throughout the duration of the experiments.

RESULTS

Yolk Content of I CGG and I* CSA in Sequentially Laid Eggs* The protein-bound I* content of yolks of eggs laid by chickens which had received a single intravenous dose of I* CGG or I* CSA is shown in Fig. 1. The blood volume determinations on these hens and the total amounts of I* proteins present in all yolks studied are shown in Table I. The blood volumes of chickens were found to approximate 7 per cent of the total body weight. The difference between this figure and the 8 per cent generally accepted for many

species of animals is probably accounted for by the weight of the chicken feathers. Three to four times more of the injected I* CGG than of the I* CSA appeared in the yolks. One day after injection of either I* CGG or I* CSA, radioactivity was detectable in egg white, but none of this radioactivity was protein-bound and was probably free iodide liberated by catabolism of labeled protein and secreted by the oviduct. Peak levels of non-protein–bound I* appeared in the whites of eggs laid 2 days after in-

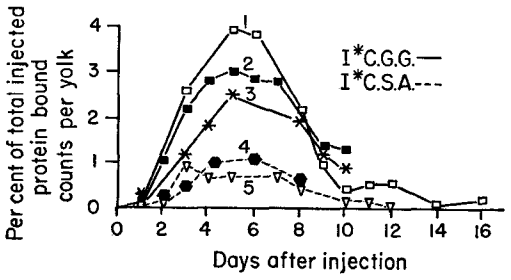

FIGURE 1. I* CGG and I* CSA in yolks of eggs laid after a single injection of labeled serum protein.

TABLE I

I* CGG AND I* CSA CONTENT OF SERUM AND EGG YOLK

Chicken No.	Protein injected	Weight of chicken	Blood volume: Weight × 100‡	Average yolk volume ml ± standard deviation	No. of eggs counted	Total I* protein injected found in all yolks counted
		gm.				*per cent*
1	I* CGG	1850	6.72	14.0 ± 0.5	11	15.58
2	I* CGG	2030	6.9	16.9 ± 1.1	8	16.25
3	I* CGG	1670	7.14	14.0 ± 0.7	6	9.53
4	I* CSA	1660	7.12	14.0 ± 0.7	8	4.15
5	I* CSA	1800	7.0	14.0 ± 0.9	6	3.15

‡ Average blood volume 7.1 ± 0.3 per cent of body weight.

jection of the labeled proteins and in the yolks of eggs laid 6 or 7 days after injection.

Autoradiographs made of yolks laid 3 days after injection of I* CGG showed marked concentration of I* in the periphery of the yolk, while autoradiographs of yolks laid 9 days after injection showed a definite concentration of I* in the centers of yolks with a lesser amount in the periphery. The concentration of I* in these repective areas indicated that diffusion through the yolk emulsion occurs very slowly.

Content of I CSA and I* CGG in Developing Ovarian Yolks* The protein-bound I* content of yolks from ovarian ova of hens which received a single

TABLE II

PROTEIN-BOUND I* CONTENT OF OVARIAN OVA FROM HENS WHICH RECEIVED INJECTIONS OF I* CGG OR I* CSA AND WERE SACRIFICED IN 24 HOURS

Yolk volume	Diameter of ova	Surface area of ova	Protein bound I*	Protein-bound I* counts in yolk/cm² of ovum surface area
		Chicken no. 6 I* CGG		
ml	*cm*	*cm²*	*counts/ovum*	
14.2	3.02	27	122,000	4,500
10.5	2.72	23.2	142,000	6,140
3.3	1.84	10.7	75,200	7,250
1.4	1.42	6.35	44,000	6,930
0.3	0.834	2.18	7,630	3,500
0.206	0.734	1.64	3,320	2,030
0.103	0.58	1.06	1,005	950
0.056	0.474	0.70	692	982
0.023	0.36	0.40	243	597
		Chicken No. 7 I* CGG		
14.2	3.02	28.5	84,500	2,970
11.4	2.78	25.2	88,000	3,500
7.0	2.39	17.7	92,000	5,200
3.1	1.84	10.5	60,500	5,760
1.3	1.354	4.92	27,800	5,630
0.45	0.95	2.82	7,180	2,542
0.15	0.66	1.36	750	552
0.15	0.66	1.36	740	543
0.15	0.66	1.36	510	380
		Chicken No. 8 I* CSA		
15	3.05	29.2	66,000	2,260
11	2.75	23.8	72,500	3,080
7.2	2.39	17.8	69,500	3,920
3.3	1.84	10.6	36,400	3,440
1.5	1.42	6.35	14,500	2,290
0.435	1.54	2.8	3,100	1,110
0.15	0.66	1.37	186	137
		Chicken No. 9 I* CSA		
17.5	3.22	32.6	29,700	910
15.4	3.08	30.0	55,700	1,860
12.3	2.86	25.7	80,000	3,110
8.4	2.50	19.8	76,000	3,820
2	1.56	7.65	16,400	2,140
0.77	1.13	4.04	6,350	1,570
0.25	0.78	1.9	503	265
0.1	0.57	1.04	130	126

injection of I* CSA or I* CGG and were sacrificed in 24 hours is shown in Table II. Results were corrected for variations in weight of birds and number of counts injected in order to express them on the basis of the same number of counts injected per unit weight of bird. Results indicated that considerably more gamma globulin than serum albumin is passed to the ovum *via* the ovary, and this is compatible with the results obtained from eggs laid serially after injection of these I* proteins into hens. The passage of both gamma globulin and serum albumin increases with an increase in the size of the ovarian ova except for those ova within 2 to 3 days of ovulation. The protein-bound I* in yolk, expressed as a function of surface area of the ovum, is shown graphically in Fig. 2. Ova less than 0.5 ml in volume receive less serum proteins than might be anticipated from their proportional size. As the volume

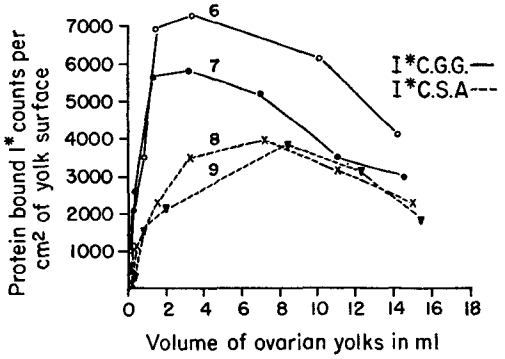

FIGURE 2. Passage of I* CGG and '* CSA to ovarian yolks during first 24 hours after injection.

of the yolk increases, there is a rapid increase in the amount of labeled protein transferred per unit of ovum surface area and thus per unit area of the ovarian follicular epithelium surrounding the developing ova. The maximal serum protein transfer by the follicular epithelium occurs in ova 3 to 4 days prior to ovulation when the volume of the ova is 3 to 8 ml. In each chicken more I* CGG than I* CSA is transferred/cm² ovum surface to each of the six largest ovarian ova. Sections of the epithelial linings and stromal walls of ovarian ova 0.3 cm and 1.4 cm in diameter are shown in Figs. 3 and 4, respectively. The follicular epithelium surrounding the larger ovum is flatter and thinner than that surrounding the smaller ovum, and the epithelium around the larger ovum is apparently transferring the larger amount of serum protein per unit of surface area. Thus, a morphologic change in the ovarian follicular epithelium of the ovary was observed during that period of development of the ova when an increase in transport of certain serum proteins occurred.

The preferental transport of I* CGG over I* CSA into ova appears to be more marked in the sequentially laid eggs (Table I) than in the ovarian ova (Table II). This difference may be due in part to biological variation in the birds studied and in part to some variation in time of sacrifice of the hens supplying the ovarian yolks. The greater transport of CGG to yolks as compared with transport of CSA was further confirmed by electrophoretic analysis of yolk proteins as described below.

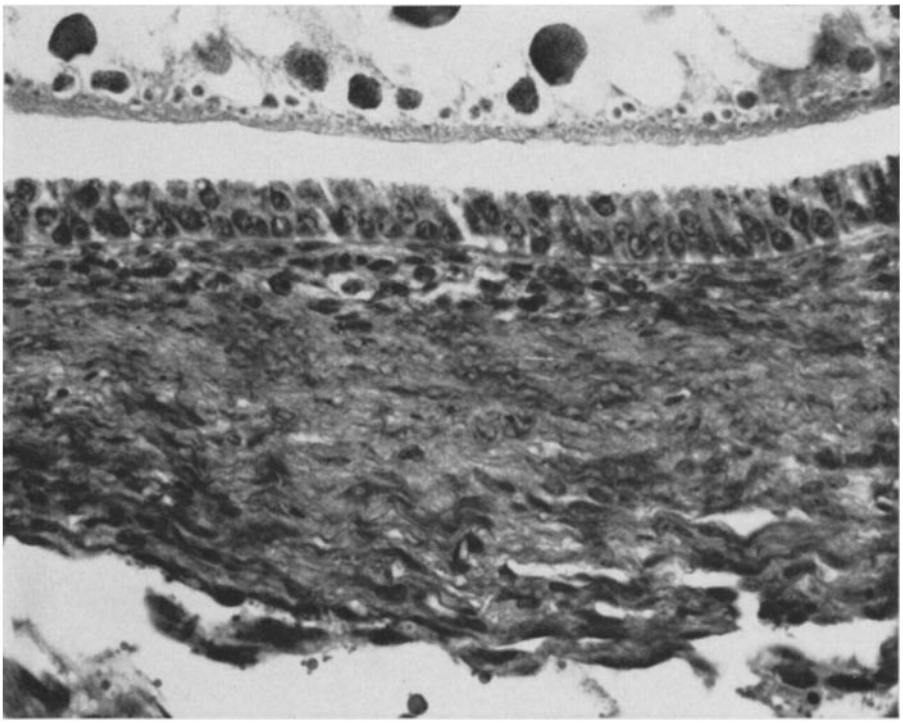

FIGURE 3. Wall of ovarian ovum 0.3 cm in diameter. This follicle is lined with pseudostratified columnar epithelium which is surrounded by ovarian stroma. Some yolk material has been lost in processing. Hematoxylin and eosin preparation. × 300.

Appearance of Antibody in Eggs Laid Sequentially after Immunization of the Hen Two chickens immunized with BSA developed peak levels of serum antibody 7 and 8 days after immunization. Anti-BSA antibody was initially detectable in yolks laid 4 days after appearance of serum antibody, and peak levels of yolk antibody occurred 5 days after peak serum antibody levels. When plotted, the antibody levels in successive yolks gave a curve similar to that obtained for the appearance of I* CGG in the yolks (Fig. 2). The investigations of yolk antibody following active immunization are reported in detail elsewhere (8).

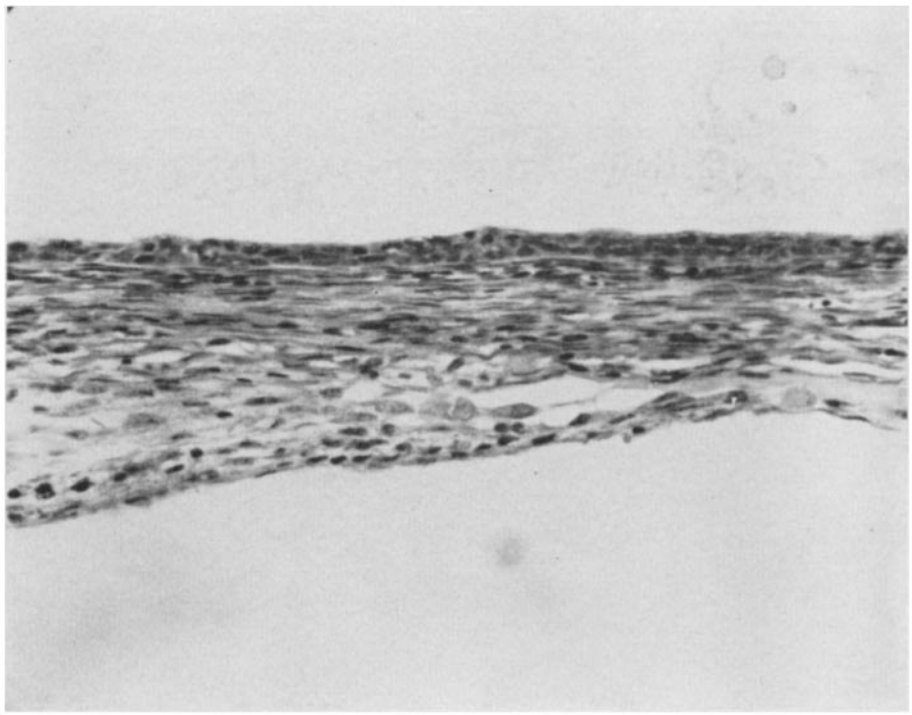

FIGURE 4. Wall of ovarian ovum 1.4 cm in diameter. Interior of ovum is at top of the photograph. The follicular lining is composed of flattened cuboidal to squamous epithelium. The majority of the yolk material has been lost in processing. Hematoxylin and eosin preparation. × 300.

TABLE III

HALF-LIVES OF SERUM PROTEINS IN LAYING HENS AND CHICKS IN HOURS

Protein	No. of birds	Half-life in laying hens	Half-life in 1 to 7 day old chicks
Serum albumin	5	66.5 ± 6.3‡	
Serum gamma globulin	4	35.0 ± 7.0	
Serum albumin	10		42.0 ± 7.5
Serum gamma globulin	10		72.0 ± 9.0
Anti-BSA antibody	6	51.0 ± 12.2§	

‡ Standard deviation.

§ Decline in serum antibody concentration after primary response.

Half-Lives of Homologous Serum Albumin and Gamma Globulin For comparative purposes, the half-lives of these proteins were determined in adult laying hens and in 1 to 7 day old chicks. The results are shown in Table III. The rate of turnover of albumin is slower in the hen than in the chick, while

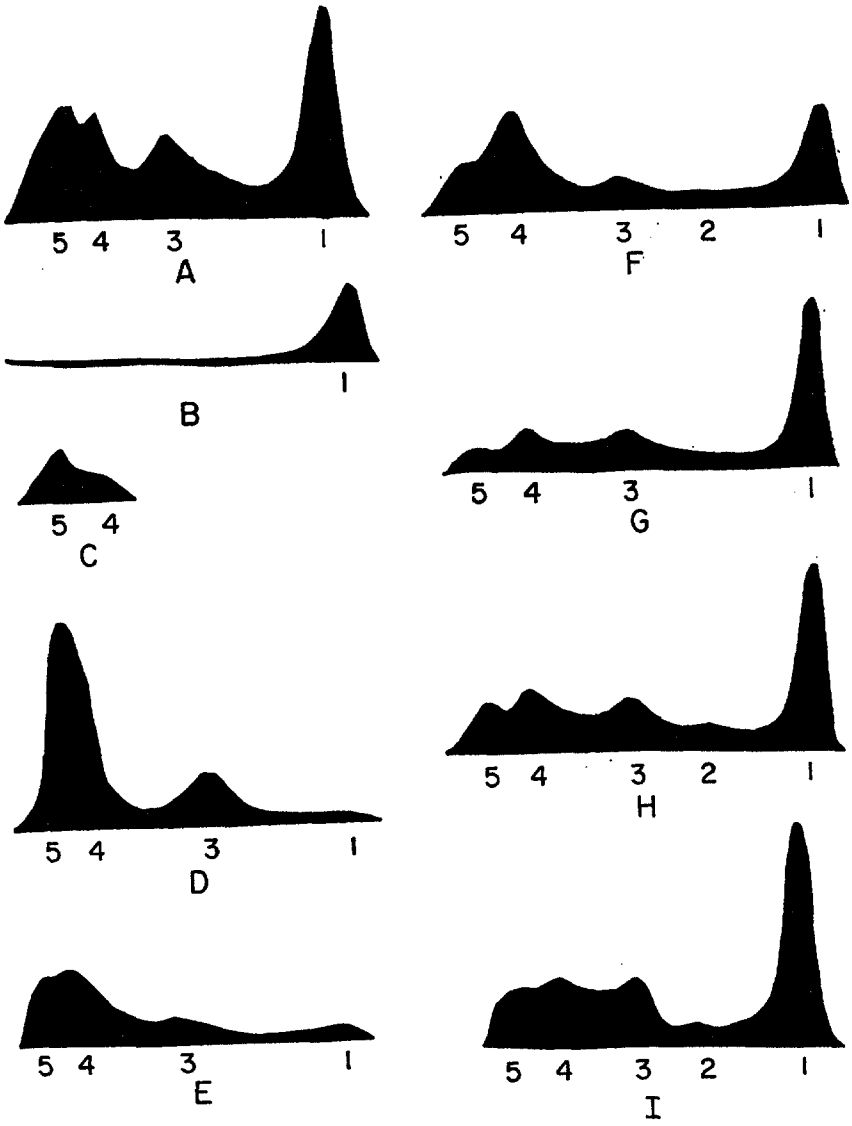

FIGURE 5. Paper electrophoretic patterns of white Leghorn chicken serum proteins. A, normal laying hen. B, serum albumin fraction of hen serum. C, gamma globulin fraction of hen serum. D, saline extract of unfertilized yolk. E, fetal chick 2 days before hatching. F, 2 day old chick. G, 15 day old chick. H, 32 day old chick. I, 58 day old chick.

the rate of turnover of gamma globulin is more rapid in the hen than in the chick. Rates of fall of chicken serum antibody levels were estimated from the decline in anti-BSA serum antibody in laying hens immunized with a single injection of BSA (8). The decline in antibody level is slower in adult chickens

than the decline of circulating I* CGG, which could be explained by continuing production of antibody after peak levels have been reached.

Electrophoretic Patterns Fig. 5 shows representative electrophoretic patterns of serum of the adult laying hen (A), albumin fraction of hen serum (B), gamma globulin fraction of hen serum (C), saline extract of yolk (D), and serum of maturing chicks (E–I). The protein concentration of the samples shown by electrophoresis is listed in Table IV. The numbered peaks of migrating proteins (Fig. 5) may be compared with similar peaks in patterns of mammalian species. The fastest migrating protein, No. 1, corresponds to

TABLE IV
PROTEIN CONCENTRATION OF SERA AND SERUM FRACTIONS SHOWN IN FIG. 5

Sample	Protein
	mg/ml
A. Adult chicken serum	41.0
B. Gamma globulin fraction	4.5
C. Albumin fraction	11.0
D. Yolk extract	22.0
E. 19 day chick embryo	16.9
F. 2 day chick	33.8
G. 15 day chick	36.4
H. 32 day chick	34.1
I. 58 day chick	36.0

the albumin; the slowest protein, No. 5, corresponds to gamma globulin. Peaks No. 2, 3, and 4 may be analogous to alpha 1 globulin, alpha 2 globulin, and beta globulin, respectively, in mammalian serum protein electrophoretic patterns.

DISCUSSION

The rate of catabolism of serum proteins in adult laying hens is more rapid than in most mammalian species studied (9). The half-life of homologous gamma globulin in the hen is most comparable to that in the mouse. The metabolic rate of the host generally correlates with half-lives of its serum proteins and the rapid rate of metabolism of the chicken may largely explain the short half-lives of these proteins.

The effect of maternal transport of CGG or CSA to the yolk on the metabolism of these serum proteins in the hen can be appreciated by considering the amounts of these proteins found in sequentially laid eggs after a single injection of the I*-labeled proteins. For example, 10 to 16 per cent of the total amount of I* CGG injected is found in the subsequently laid eggs

(Table I) while the remaining 84 to 90 per cent is apparently lost by catabolism.

The half-life of gamma globulin in the chick is approximately two times as long as in the adult hen, which corresponds to the difference in the half-lives of gamma globulin in neonatal and adult rabbits (10). However, the half-life of serum albumin in young chicks is shorter than the half-life in the adult hen, a situation not paralleled in the rabbit (10).

The passage of serum proteins from the circulation of the hen to the yolk provides the developing fetus with a supply of these proteins. This transport to the ovum of serum proteins as well as other yolk materials is most likely a function of the follicular epithelium of the hen's ovary. This epithelium is formed from cells derived from the epithelium covering the gonad of the chicken embryo (11). During maturation of the ovum in the ovarian follicle this epithelium proliferates and undergoes a change in morphologic appearance characterized mainly by a decrease in the height of the follicular epithelium. This flattening of the epithelium occurs before the ovum is 0.6 cm in diameter or approximates a volume of 0.1 ml (11). As seen from Table II and Figs. 2 to 4, it is after this epithelial change occurs that the transport of gamma globulin across the epithelium is accelerated. The maximum transport of gamma globulin per square centimeter of yolk surface occurs in smaller ova than those showing maximum transport of serum albumin (Table II and Fig. 2). When the ovum enters the last growth phase in the chicken ovary, a non-cellular membrane, the vitelline membrane, appears between the edge of the ovum and the follicular epithelium of the ovary (11). During this last phase of growth of the ovum, the yolk volume does not increase as rapidly as before, and there is a decrease in the amount of serum albumin and gamma globulin that is passed to the ovum.

The time of appearance and concentration of the I*-labeled homologous serum gamma globulin and serum albumin in yolks laid after intravenous injection of the hens (Fig. 1) reflects the growth and protein transport features of the developing ovarian ova described above. The eggs laid within 1 day after injection of the hens with labeled protein contained no protein-bound I* since they were already in the oviduct at the time of injection. Yolks of eggs laid 2 to 5 days after injection of the hens contained increasing amounts of I* proteins. At the time of injection of the hens, these yolks were in ovarian follicles at the stages of development when protein transport to ovarian ova is greatest (Fig. 2). Eggs laid more than 6 days after injection of hens showed progressive decline in the yolk I* protein content (Fig. 1). At the time of injection, these yolks were in ovarian follicles at stages of development prior to the period when I* protein transfer to ova is greatest. In addition, the I* protein deposited in these ova during initial periods of high blood concentration is diluted by subsequent deposition of yolk material after circulating

I* protein levels have declined. The longer plateau of the I* CSA curve as compared with the I* CGG curve (Fig. 1) is the result of the longer half-life of I* CSA and, therefore, more prolonged exposure of the ova to higher circulating levels of I* CSA during ovarian development of these yolks.

The transport of gamma globulin to the chick *via* the yolk may be contrasted with other methods of passive maternal immunization of the fetus or neonate in different species. Passive maternal immunization of rabbit, guinea pig, and human fetuses occurs *in utero* and is presumably regulated by extraembryonic non-maternal tissues as in the case of the yolk sac splanchnopleure in the rabbit (12). In the ungulates the acinar epithelium of the udder specifically concentrates maternal gamma globulin into the colostrum for transfer to the offspring through the gastrointestinal tract after birth. In dogs, rats, and mice both methods of transfer take place (12). In the hen, the passage of gamma globulin and antibody occurs entirely in the ovary since all yolk material is deposited in the ovary and no I* CGG or antibody was detected in egg white, which is formed in the oviduct, and the egg shell is already formed prior to entrance of the egg into the chicken cloaca. The preferential passage of gamma globulin over serum albumin to the yolk may be compared with similar observations on the maternal passage of serum proteins from the udder of the cow into its secretions (13), since it has been reported that gamma globulin is preferentially concentrated into lacteal secretions over all other serum proteins. During colostrum formation in the cow, gamma globulin is concentrated in the colostrum 100 times more than is serum albumin. Thus, the follicular epithelium of the chicken ovary performs the same service as the acinar epithelium of the cow's udder and the extraembryonic tissues of rabbit and man, by preferentially transferring antibody protein from the maternal circulation for passive immunization of the fetus.

The electrophoretic analyses (Fig. 5) of serum proteins of the adult hen and saline extract of yolk provide confirmation of the results described above showing the proportionately greater transport of CGG than CSA to yolks. In the saline extract of yolk (Fig. 5D), a considerably greater amount of gamma and beta globulin is present than albumin.

In the circulation of the fetal chick, there is a preponderance of gamma and beta globulins. The fetal chick 2 days before hatching (Fig. 5E) has less albumin than these globulins. The 2 day old chick (Fig. 5F) shows an increasing amount of serum albumin. This might result from a more rapid synthesis of albumin than gamma and beta globulin by the young chick or a preferential mobilization of albumin from the yolk to the circulating during the later stages of incubation. Fifteen days after hatching (Fig. 5G), the amount of albumin in the circulation far exceeds the amount of gamma globulin. The gamma globulin level is increased in the 32 day old chick

(Fig. 5H) indicating gamma globulin synthesis prior to this age. In agreement with this, antibody formation has been shown in almost all birds by the age of 35 days (14).

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