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A STUDY OF THE ELECTROPHORETIC PATTERN OF THE SERUM PROTINS

OF INDIVIDUAL CHICKEN DURING DEVELOPMENT

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Introduction

A number of workers have investigated the electrophoretic pattern of the seum proteins of chicken in various periods of development during the embryonic, as well as the post-hatching stages, using free boundary (Moore *et al.*, 1945; Marshall and Deutsch, 1950; Brandt *et al.*, 1951; Heim and Schechtman, 1954) and paper electrophoresis (Common *et al.*, 1953; Vanstone *et al.*, 1955; Hradec and Lemez, 1954). In general, the findings of these various authors are not in complete agreement as to the number and the relative concentrations of the various fractions found in the chicken embryo. Thus Moore *et al.* (1945) reported the presence of only two to three fractions in the early embryonic stages, while Marshall and Deutsch (1950) found between eight to nine fractions during the same period of development. The lack of uniformity in the results obtained can partly be explained by the use of different buffers applied. The early ultracentrifugal studies of Moore *et al.* (1945) indicated that the serum of chicken embryo was made up of a single molecular component. Marshall and Deutsch (1950) demonstrated, however, that the system is complex. By the use of free bounhary electrophoresis, Marshall and deutsch (1950) showed that aside from components that move ahead of albumin, almost all other components in the serum of embryo corresponded to the protein fractions of the adult chicken.

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Due to its simplicity and the need for small material, paper electrophoresis has gained wide application in recent years for the study of the electrophoretic pattern of chicken serum in various stages of development in normal (MCKINLEY *et al.*, 1953; COMMON *et al.*, 1953; MCCULLY *et al.*, 1959) as well as in abnormal cases (WOSTMAN and GORDON, 1958; YASAIR *et al.*, 1959). VANSTONE *et al.* (1955) have shown that the 14 and 18 day old embroyos contain four protein fractions in their serums which correspond to albumin, alpha-2 and beta-globulins plus a component which moves ahead of albumin. Alpha-1 and gamma-globulin fractions appear only at the time of hatching. HRADEC and LEMEZ (1954) investigated the proteins of embryos beginning from the 8th day incubation until the time of hatching. Their findings indicated that aside from fraction 6 (alpha-2 globulin), all other components which are normally present in the adult chicken, are absent from the serum of embryos. According to their findings, albumin appears on day 19 of incubation. The pre-albumin component which begins to appear on day 12 of incubation and also fraction 1 and fraction 3 (a lipid carrier) disappear at the time of hatching.

The present work was undertaken to make further investigation upon the paper electrophoretic behavoir of the developing chicken embryo. Moreover, the behavior of the chicken serum was also studied during the few weeks of the post-hatching period; the serum of each individual chicken, which was bled repeatedly for a period of six weeks, was compared throughout this period of development.

MATERIALS AND METHODS

New Hampshire chickens of the embryonic and post-hatching stages were used throughout these experiments. The chickens were mintained on a diet consisting of the following: wheat 40%, barley 20% meat 5-6%, bran 15%, defatted cotton seed 8%, bone powder 2-3%, dry fish powder 3%, skimmed milk 5%, and salt 0,5%. In addition alfalfa was added to their meals.

The eggs were incubated at 39.5° C. Blood was drown from the chorioallantoic vein. The sera of at least 15 embryos were pooled for each experiment. The amount of serum taken from each embryo varied between 0.05 to 0.20 ml., depending on the age of the embryo. Blood was taken from the heart in newly hatched chicks, and from the wing vein of older fowls. The chickens were starved for 18 hours before bleeding.

Electroporesis: Paper electrophoresis was carried out on Whatman No. 1 filter paper, using Elphor apparatus as described by GRASSMAN and HANNIG (1952). Fifteen microliters of serum was introduced to each strip

of filter paper in case of older birds and twenty to thirty microliters in case of the serum from embryos. Electrophoresis was performed for 16 hours, at a voltage of 110 and 2 milliamperes. The buffer used was a mixture of sodium veronal and sodium acetate of 0.1 ionic strength and pH 8.6. The paper strips were stained with amido black 10 B, according to the procedure of GRASSMANN *et al.* (1951). A thorough washing of the stained strips was carried out in a mixture of methanol and glacial acetic acid (90 parts and 10 parts respectively), for a period of six hours, with constant stirring. Densitomener reading of the paper strips was carried out by a Photovolt densitometer, after making the strip transluscent with a mixture of alphabromonaphthalene and paraffin, having a refractive index similar to that of the strips.

RESULTS

Table 1 shows the relative percentage change in the areas of the various protein fractions which take place throughout the developmental period, in the embryonic as well as the post-hatching stage, irrespective of sex. The serum pattern is divided into five main areas, which in the order of decreasing mobility are a pre-albumin, albumin, alpha-globulin, beta-globulin and gamma-globulin. The further resolution of the globulin areas into sub-fractions are made as alpha-1 and alpha-2, beta-1 and beta-2, gamma-1, gamma-2 and gamma-3. The results given for each experiment concerning the embryos, are of the pooled sera, wheras the values given for the serum of the chicken during the post-hatching stage, are from individual birds. The standard deviations are given only for those experiments in which number of analysis exceed four experiments, as is observed in the table. Since the resolution of the globulin areas into respective subfractions were irregular, the standard deviation is given only for the sum of each area and not for each sub-fraction separately.

The representative pattern of the chicken serum throughout the development is shown in Fig. 1. The patterns of the post-hatching period shown in this figure, are from a single chicken (No. 721), except for the one year old cock. As it is noticed the zone in front of the albumin persisted for several weeks and then disappeared. No pre-albumin was noticed in any one of the one-year old roosters which were examined.

DISCUSSION

MARSHALL and DEUTSCH (1950) were able to distinguish 9 components in the embryonic sera of chicken, of which 6 fractions (fractions 1 through 6) corresponded in mobility to the gamma-glomulin and the albumin areas

Table 1. Percentage Composition of the Serum proteins of Developing Chicken

Age	No of Analysis	Pre-albumin	Albumin	Globulins						
				Alpha-1	Alpha-2	Beta-1	Beta-2	Gamma-1	Gamma-2	Gamma-3
10 days of incubation	1		24–3			34–2		27.4	13.7	
11 〃	2	5.0	41–7			25.0			20.0	
14 〃	5	6.0(±2.2)	37.0(±9.5)			34.7(±5.6)		21.4	4.0(±7.5)	
15 〃	7	2.4(±2.6)	40.2(±5.3)			26.0	3.1(±5.1)	25.0	2.7(±9.1)	
16 〃	4	8.9	31.5			19.0	7.9		30.5	
17 〃	3	4.9	46.9			19.8	2.9		23.3	
18 〃	2	3.6	42.6		0.7		17.8	30.0	1.7	
19 〃	2	20.0	45.5		4.9		9.9		29.7	
20 〃	2	15.3	41.9	3.0	0.5	8.9	1.0		30.4	
2 days post-hatching	5	11.1(±2.1)	31.7(±1.9)	5.2	6.0(±2.9)		9.6(±2.4)	32.8	3.3(±1.5)	
4 〃	6	10.8(±6.0)	33.0(±7.5)	5.1	7.4(±1.6)		9.6(±1.1)	30.7	2.4(±2.2)	
6 〃	5	10.9(±1.9)	37.7(±3.7)	4.2	7.3(±2.0)		10.1(±1.9)	25.7	4.9(±3.1)	
8 〃	2	15.3	30.2	3.3	8.7		10.2	27.6	5.0	
10 〃	2	12.1	35.8		9.6		13.6	22.6	5.3	
14 〃	10	7.0(±3.2)	41.2(±5.3)	7.8	1.9(±2.1)	13.3	1.4(±2.5)	21.0	6.0	0.5
18 〃	6	8.4(±2.4)	40.9(±8.1)	6.0	4.5(±2.2)	12.4	1.7(±4.3)	19.5	8.3(±3.5)	
20 〃	2	7.7	17.2		9.1		12.6	18.3	6.3	
24 〃	4		48.2	6.5	1.9	12.3	2.0	16.8	8.5	0.3
28 〃	4	9.2	42.1	5.2	1.9	12.7	2.0	17.9	7.7	0.6
30 〃	1	9.9	38.9	5.2	7.8		10.9	18.5	9.1	
32 〃	2		35.9		8.6	12.7	3.4	20.2	7.1	
38 〃	1		30.5		8.5	9.7	4.9	20.7	14.6	
40 〃	1		57.5		5.0		7.5	20.0	10.0	

The figures in parentheses refer to the standard deviations (see text).

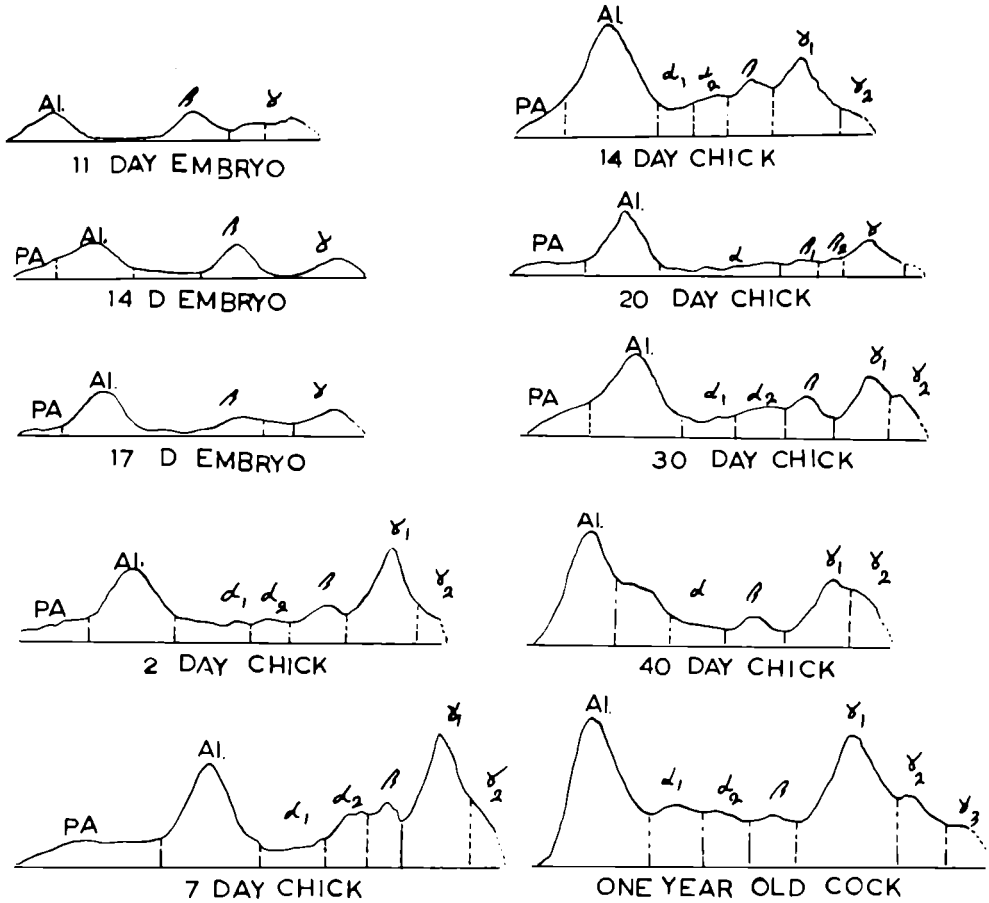

Fig. 1. Representative paper electrophoretic pattern of the serum protein of devetoping chicken.
Pa: Pre-albumin, Al: Albumin

of the adult chicken, respectively. The three other components (7 through 9) comprising more than 30 per cent of the total embryonic serum, moved ahead of albumin. HEIM and SCHECHTMAN (1954), also using moving boundary electrophoresis, demonstrated results similar to those of MARSHALL and DEUTSCH, although the lesser percentage of the fast moving components found by these authors might be due to the fact that they used buffer different from that used by MARSHALL and DEUTSCH. The use of various buffers has indeed resulted in considerable differences in the electrophoretic

pattern of animal sera. MOORE *et al.* (1945), using phosphate buffer of PH 7.4 did not report presence of the prealbumin components in the embryonic sera of chicken.

By the application of paper electrophoresis, VANSTONE *et al.* (1955) demonstrated the presence of four components in 14 and 18 day old embryos, consisting of a pre-albumin, albumin, alpha-2 and beta globulin. Alpha-1 and gamma-globulin appeared to be present in a day old chick, according to these authors. The results presented in this paper for the serum embryo are in agreement with the findings of VANSTONE *et al.* (1955). Four components were present in the serum of the embryos corresponding to a prealbumin, albumin, beta and gamma-globulins. The alph-globulin began to appear in the later days of incubation. The resolution of the gamma-globulin band into two fractions was not very clear until after hatching. The resolution of the other two globulins into alpha-1 and alpha-2 and beta-1 and beta-2 was quite irregular in most of the patterns, although the resolution of the beta region into two fractions was more distinct from the second week after hatching. Occasionally a slow moving component which was designated as gamma-3 globulin could be noticed during the post-hatching period. This component is clearly seen on the pattern of a year old rooster, as is shown in F.g. 1.

Although the various fractions observed on zone electrophoresis correspond with free boundary electrophoresis, the high percentage of the components moving ahead of albumin observed by some authors (MARSHALL and DEUTSCH, 1950) using free boundary electrophoresis cannot be seen on paper electrophoresis.

CROOK *et al.* (1954) have suggested that there can be little correlation between the results of zone electrophoresis and free electrophoresis. They regard the similarity between the results of zone electrophoresis and those of free boundary as a "coincidence". The difference observed between the results obtained by free boundary electrophoresis of the embroynic sera and those obtained by paper, particularly in regard to the components that move ahead of albumin might confirm the suggestion of CROOK *et al.* The results obtained by HRADEC and LEMEZ (1954) do not agree with the results obtained by other investigators. These authors find that with the exception of one component (component 6, apparently equivalent to alpha-globulin), none of the components found in the embryonic sera corresponds with those components present in the serum after hatching. Their results indicate that the serum proteins of 20 day old embryo consist of fractions 1, 3, 6, 8, and 9, whereas the serum proteins of a day old chick consists of fractions 2, 5, 7, and 8. Fraction 8, corresponding to albumin appears only

on day 19. Although these authors did not actually measure the rate of mobility of these various fractions, the consideable change in regard to the mobility of these components in such a short time seems unlikely.

As shown in Fig. 1, the albumin component did not behave as a single fraction and continued to produce a zone moving in front of it, behaving similarly to the pre-albumin present in the serum of the embryo. The tendency to produce a zone continued for several weeks, but could not be observed in older birds. Whether or not the pre-albumin noticed in the post-hatching period is similar to the pre-albumin present in the serum of the embryo, is not clear. Staining for lipo-protein did show, however, that the zone is a lipoprotein (AMIN, unpublished observations). It has been pointed out (SCHJEDE, 1956) that these fast moving components may not be qualitatively distinct proteins. A tryptophan-rich component which moves ahead of albumin has been characterized in human serum (SCHULTZE *et al.*, 1956). The biological significance of this pre-albumin remains to be determined, although binding of thyroxine by a pre-albumin has been demonstrated (INGBAR, 1958). Whether or not the pre-albumin observed in chicken serum compares with the pre-albumin identified in human serum cannot be determined at present. The pre-albumin characterized in human serum is shown to be glycoprotein in nature (SCHULTZE, 1958).

Feeding and genetic variations might both be considered as factors causing this stretch in front of albumin, since electrophoretic pattern of the sera of a number of incubated eggs and young chicks of native origin purchased from local hatchery did not show the pre-albumin during the post-hatching stage.

SUMMARY

Paper electrophoretic behavior of the serum proteins of the developing chicken has been investigated during both the pre-hatching and post-hatching periods, beginnings from the 10th day of incubation up to the 6th week after hatching. Four fractions could be distinguished during the pre-hatching period which were designated as a pre-albumin, albumin, beta-globulin and gamma-globulin. Alpha-globulin appeared in the last days of incubation. The pre-albumin component did not disappear immediately after hatching, but persisted for several weeks and then disappeared.

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