BIOCHEMICAL STUDIES ON THE EGG FORMATION IN THE DOMESTIC FOWL. II. THE ELECTROPHORETIC INVESTIGATION OF THE LAYING HENS' SERUM.

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II. THE ELECTROPHORETIC INVESTIGATION OF THE LAYING HENS' SERUM*.

By

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The adaptation of the electrophoretic methods to study the changes of protein components in the blood serum is well known. Only a few works, however, have dealt electrophoretically with the variation of the avian serum during the reproductive activity, and those works are mostly directed to the analysis of the serum of estrogenized birds (1-5).

On the other hand, considerable attention has long been paid to the problem of the mobilization of large amounts of proteins, lipids and other substances at the time of egg production. The mobilization of those materials was also thought to be necessary to mature the egg yolk (6,7). In a previous paper, the senior author (H. S.) reported the characteristic changes of the liver and serum composition at the time of egg formation, i. e., the occurrence of the phosphoprotein in these tissues and the increase in the protein content of the laying serum (8). From these findings, it was possible to surmise the apparent differences of electrophoretic patterns of the laying hens' serum. It was from this view that the authors became interested in the subject and the present studies were designed to find the differences of the electrophoretic patterns, with special reference to the differences in pattern areas in the normal laying hens' serum as compared with those of non-laying hens, cocks, and immature cockerels.

Materials and Methods

The breeds of chickens used were White Leghorn and Rhode Island Red. The serums were obtained from the blood of the following groups: 1) laying hens, 2) non-laying hens, 3) cocks and 4) immature cockerels. Particular attention was paid to select hens at the optimum point of the laying cycle, and the egg laying rhythm was carefully checked by the trap nest. For non-

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laying hens, two brooding hens were used, because their ovaries were almost completely inactive regarding egg formation.

The techniques of the electrophoresis were carried out according to the standard method established by the Japanese Association of the Studies for Electrophoresis (9, 10). After the serums were prepared from the blood clots, they were diluted to about 2 per cent in protein content, checking with a hand refractometer (Hitachi, Ltd.); and then they were dialized against buffer overnight in the refrigerator. Experiments were conducted with the Tiselius apparatus (HT-A or HT-B types, Hitachi Ltd., Tokyo) at a current of 8–9 mA for 45–60 minutes. Phosphate buffer (pH=7.6, ionic strength=0.144) was used in this investigation, but the veronal buffer (pH=8.4, ionic strength=0.1) was also used to compare with the results of the laying hens' serum in the phosphate buffer. The composition percentage of the pattern was derived from the ascending pattern by the weighing methods, because the figures of this pattern were separated distinctly, in particular, the appearance of component 1 (fastest moving fraction), as will be mentioned below.

As to the nomenclature of the fractions, it was most common to number from 1 to 6 according to the mobility, but McKinley and his co-workers (11) suggested the tentative correlation between the components described by Clegg and his colleage (12) and their own results, as follows:

component 1 —— (fastest moving fraction)
component 2 —— albumin fraction
component 3 —— α-1- globulin fraction
component 4 —— α-2- globulin fraction
component 5 —— β- globulin fraction
component 6 —— γ- globulin fraction

We decided to use the above names for the fractions in our report.

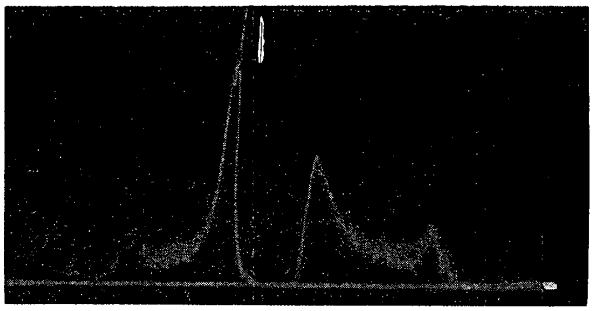
Results

The results of the electrophoretic analysis are summarized in Table 1 and Plates I and II.

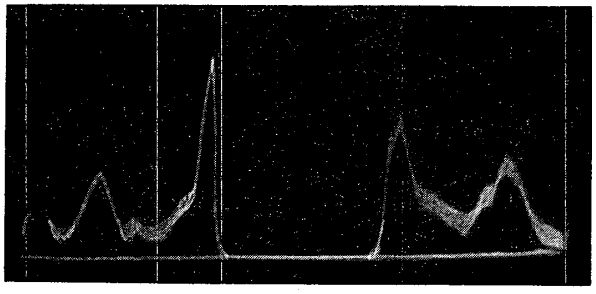
Table 1. Electrophoretic pattern area of the fowl's serum

Buffer	Chick group	Case no.	Component, per cent					
			f-frn.	al-frn.	α_1-frn.	α_2-frn.	β-frn.	γ-frn.
Phosphate pH=7.6	Immature cockerels	2	—	39 5	11.5	8.5	13.6	27.3
	Cockes	4	—	38.4±1.4*	9.1±1.7*	7.5±1.7	12.6±1.8	32.3±2.5**
	Brooding hens	6	—	39.4±1.4*	9.4±1.4**	7.7±1.2	9.5±0.5†	33.1±8.6**
	Laying hens	9	4.8±1.4	21.1±4.2	6.?±2.3	78.±3.1	11.0±2.9	49.0±9.7
Veronal pH=8 4	Laying hens	7	3.6±1.3††	27.2±4 8	7.4±1.5	7.9±4.9	11.8±4.4	43.0±11.1

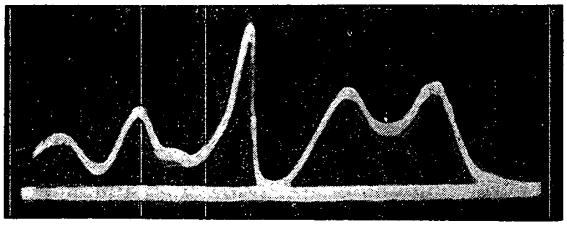
* α=0 05 } Comparing with the value of the laying hense } Significant at the level of,
** α=0 01 }
† α=0 05 Comparing with the value of the cocks
†† In four cases out of seven, the component was detected.



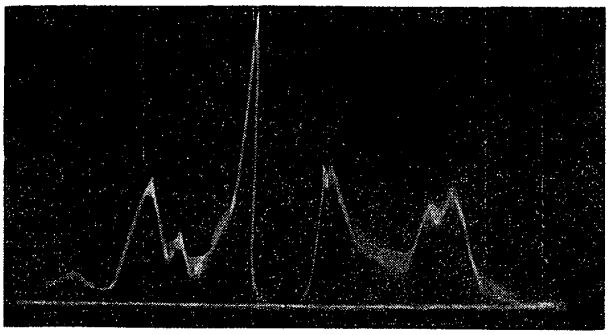
A, immature cockerels



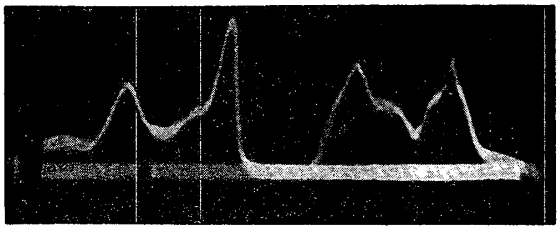
C-1, non-laying (brooding) hens



C-2



B, cocks



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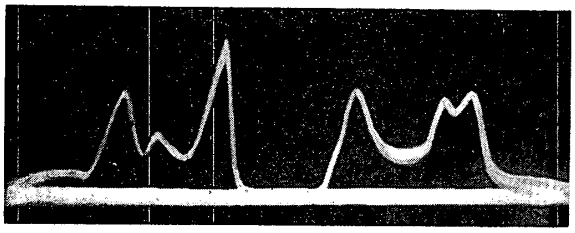
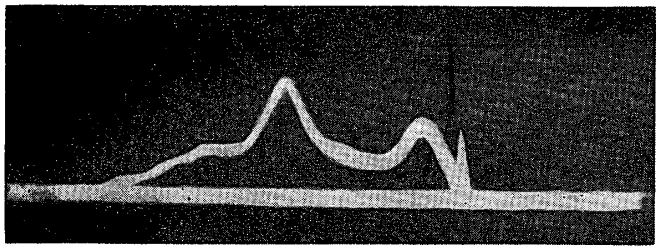
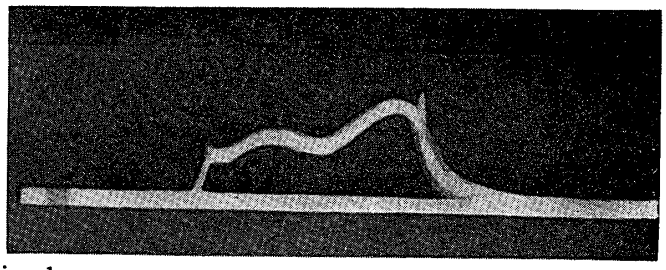


C-4

Plate I Electrophoretic patterns of the blood sera of the immature cockerels, cocks and non-laying (brooding) hens.

(as)

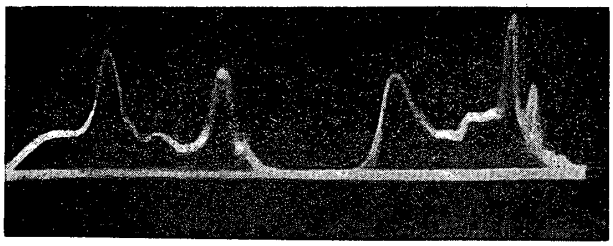


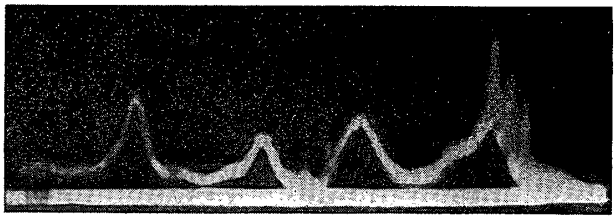


(des)

D-1 laying hens

※ ※



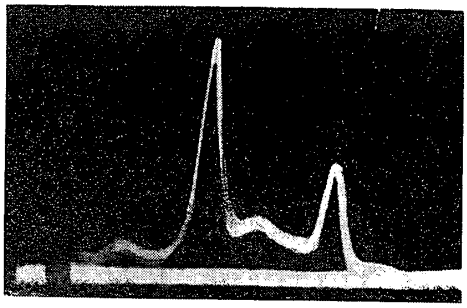


D-2 ※ D-3

D1-4 : phosphate buffer

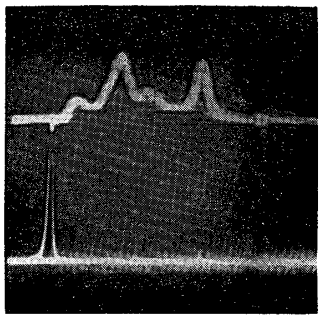
E1-5 : veronal buffer

(as)

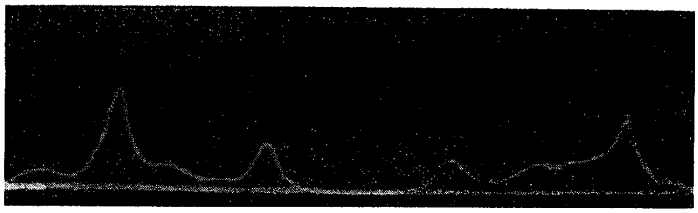


D-4

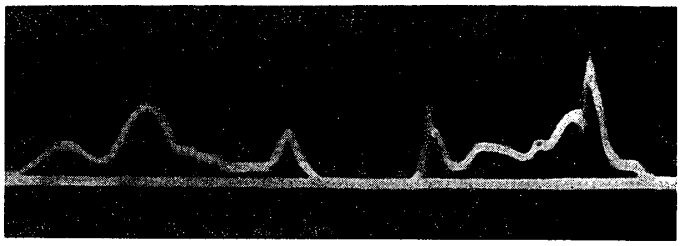
(as)



E-1 laying hens

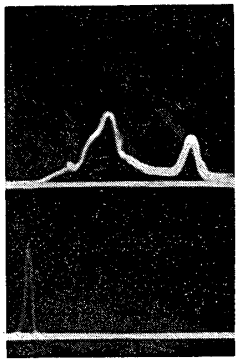


E-3

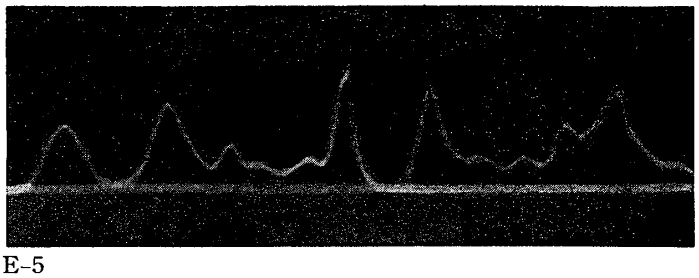


E-4

(as)



E-2



E-5

Plate II Electrophoretic patterns of the blood sera of the laying hens in phosphate and veronal buffer.

※ indicate patterns which were a little modified to make clear appearance.

The electrophoretic pattern of the domestic fowl, on the whole, would not separate so as to give a definitive reading. Some of the laying hens' serum especially did not give distinct pictures, because of the thick turbidity caused by the high content of lipids. Comparing the pattern produced by different buffers, it was noticed in the veronal buffer that the γ-globulin separated from the salt or ε-boundary, that it was possible to measure its magnitude more accurately, and that the β- and γ-globulin were sometimes not as well defined as in the phosphate buffer in accordance to Moore's description (1). However, the mobility of the protein patterns was higher in the veronal buffer.

The serum patterns of immature cockerels, cocks and non-laying hens showed approximately the same tendencies, though there was some variation. The pattern of the non-laying hens serum was essentially the same as that of the cocks excepting only for the moderate decrease in β-globulin. The pattern of the immature cockerels was significantly low in the γ-globulin fraction (Table 1, Plate 1, A, B, and C 1-4).

In contrast, the pattern of the laying hens' serum remarkably altered from those described above: namely, 1) the occurrence of a fastest-moving component (so called f-fraction), 2) the increase in size of the slowest moving fraction (γ-globulin fraction) and at the same time the decrease of albumin fraction, and 3) the reduction of α-1-globulin compared with the patterns of cocks and non-laying hens (Table 1 and Plate II).

In the pattern of the laying hens, the f-fraction ahead of the albumin fraction was detected when using the veronal buffer as well as the phosphate buffer, but the amount of f-fraction was somewhat smaller in the veronal buffer than in the phosphate buffer. In addition, the f-fraction was found only in four cases out of seven. The existence of this fraction, however, could not be found in the patterns of the other groups at all, whenever using phosphate buffer.

The enlargement of the γ-globulin fraction was significantly clear. The pictures of this fraction frequently appeared faintly, so that their good pictures could not be taken. This might be caused by the high content of lipids in this fraction (Plate II, D-2 to D-4).

There was an another matter to be noted as a by-product of the results in this studies: There was some tendency toward variation in the pattern area of the two brooding hens used as non-laying hens; i. e., a decrease in the albumin fraction and therewith an increase in γ-globulin fraction as the brooding period progressed (Plate C 1-4). One of the other definite differences in the brooding hens' pattern was the diminution of β-globulin fraction, as was stated above.

Discussion

Several characteristic variations of the laying hens' serum were revealed by the electrophoretic analysis. Although many works have been directed toward culling evidence on the changes of serum composition at the time of egg formation or of estrogen treatment, little is known as to the exact figure of the pattern area of the normal laying hens. Because these works aimed at determining of the variation caused by estrogen administration and little reference was made regarding the egg laying rate.

As has been proved, the comparison of two electrophoretic patterns produced by different buffers was unsuitable, since the types of pattern obtained depend appreciably on the buffer used. For instance, the γ-globulin did not separate from the ε-boundry in the phosphate buffer, and the percentage of composition of the γ-globulin fraction in this buffer became large by an amount dependent on the serum dilution (1). But, the presence of the f-fraction was evident and distinct in the phosphate buffer, so that the comparison of the pattern area was drawn by use of the phosphate buffer in this examination.

While there was a considerable degree of correspondence between the results obtained and the experiments of others which will be mentioned below, these discrepancies might be ascribed to either the condition of the hens used or to the different kinds of buffer employed. In conclusion, our findings supported the view that the electrophoretic patterns of the laying hens' serum showed marked differences compared with those of non-laying hens, cocks and immature cockerels: 1) the occurrence of the f-fraction, 2) the increase in γ-globulin fraction and decrease in albumin fraction and 3) moderate reduction of α-1-globulin.

Brandt et. al. (2) stated that the f-fraction which appeared well in borate buffer was never seen when using the veronal buffer or others. He explained further that it was a success to use the borate buffer and accounted for the lack of mention of the f-fraction in other previous reports in this field. Neverthelass, the distinct figures of the f-fraction could be detected in the phosphate buffer in this study, aswell as in the observation by Moore (1). And the fraction was discovered in relatively small and variable amounts even when using the veronal buffer. Equally important in laying hens' pattern was the increase in size of the γ-globulin fraction, the reduction in size of albumin fraction, and the decrease in size of α-1-globulin. It has been suggested that these phenomena associated with the egg laying were also manifested in estrogenized fowls, even in the male (4, 5). But, as will be noted later, these characteristic changes seemed to be somewhat exaggerated in the estrogen treated chickens. When the above phenomena were noticed in the studies of estrogenized fowls, it was suggested that these physiological changes were

associated with the egg-yolk forming process. Our results of the laying hens' serum tended to confirm the previous descriptions.

Although a complete explanation of the characteristics of the laying hen's serum has not been given in previous works as yet, we will discuss the available materials including the results obtained in the studies of estrogen-treated birds, with reference to this investigation.

In this experiment using the phosphate buffer, Moore (1) was the first to notice significantly larger amounts of β-globulin in serum from an ovulating hen than could be found in serum of the non-laying hens. Later, he examined the effects of estradiol administration (0.1 mg daily for 18 days) on capons and noticed the appearance of f-fraction and a marked increase of β-and γ-globulin in those fowls. He, moreover, showed that these sex differences were largely removed by ether extration. He observed furthermore that α-1-globulin which was larger in normal cocks than in laying hens, almost always diminished immediately after the beginning of the hormone treatment. Similar results were obtained by Clegg et al. (5, 12). Judging from their figures, it may be said that the enlargement of β-globulin are more prominant in estrogenized chickens' serum than in naturally laying hens' serum. The f-fraction and β-globulin fractions were characterized by a high radioactivity, when P-32 had been fed (1 mc daily for several days). A remarkable difference was the dominant increase of γ-globulin fraction in present work forming a contrast to that of β-globulin in the results of Moore and Clegg et al.

When they applied the paper electrophoresis to the avian serum, Common and his co-workers (11, 13) were able to demonstrate much evidence about component changes to estrogenized pulletts' serum They showed that a new heavily staining fraction extended from just behind the patent or expected position of α-2-globulin back to the supposed β-globulin. This new band tended to show "tailing" which became more pronounced when the fowls had heavily been estrogenized. They named the new band as "presumptive lipovittelin." Another definitive new band was demonstrated at near the starting point by use of the methanol-adding veronal buffer but its content was not appreciably altered by Bloor's mixture extraction.

Further studies into the properties of the characteristic fractions for laying hens, especially the localization of phosphoprotein (vitellin) in the serum should be the subjects for future research.

So far as known, literatures bearing on the electrophoretic composition of brooding hens' serum war scanty. To account for the variations of albumin- and γ-globulin-fraction as the brooding period progresses, and for the marked decrease of β-globulin, nothing has yet been clarified.

Summary

Comparative studies of the electrophoretic patterns of laying hen's serum with those of non-laying hens (brooding hens), cocks and immature cockerels were carried out. Characteristic changes of the laying hen's serum were demonstrated as follows: 1) the occurrence of f-fraction moving ahead of the albumin fraction, 2) the increase in size of the γ-globulin fraction and the decrease in size of the albumin fraction, and 3) the reduction of the α-1-globulin fraction.

Brooding hens' serum showed a marked diminution in the β-globulin fraction.

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