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Nishiyama, Hisayoshi
Laboratory of Zootechny, Faculty of Agriculture, Kyushu University

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On the characteristics of the transparent fluid

II An electrophoretic study of proteins of the transparent fluid

Hisayoshi Nishiyama

The transparent fluid of the cock is the fluid which flows from its accessory reproductive organs and is added to the vas deferens semen simultaneously with the ejection of it. The fluid, then, corresponds to the secretion of the accessory reproductive organs in mammals (Nishiyama, 1955). However, in the previous paper the writer (1954) reported that the origin of the transparent fluid, unlike the secretion of accessory reproductive organs in mammals, lay in theblood.

It was of interest to investigate whether or not the protein constituents of the transparent fluid were similar to those of the blood serum of the cock. For this paper, the composition of transparent fluid was analyzed by electrophoresis and was compared with that of the blood serum of the same bird.

This investigation was carried out under the direction of Professor Masaharu Tange.

Materials and method

In the cock, the vas deferens semen is ejected from the openings of vasa deferentia and the transparent fluid flows out simultaneously with the ejection of vas deferens semen from the lymphfolds of both sides. Then, in order to collect the transparent fluid, a cock which had been obstructed from contamination of the semen in vas deferens, by means of binding the anterior vasa deferentia with surgical threads and destruction of the openings of vasa deferentia with electric cautery was used as in the previous paper (Nishiyama, 1954). The transparent fluid was collected by means of abdominal massage method (Burrows

and Quinn, 1937) from a cock mentioned above. In collection, the outside of the anus was cleansed and a mass of cotton was inserted into the rectum in order to block any contamination. Soon after the collection, dilute gelatinous substance or substances considered to be a matter similar to fibrin appeared in the collected transparent fluid, as reported in the previous paper (Nishiyama, 1955). This fibrin was removed and the fluid without fibrin was used. The amount of the transparent fluid which was able to be collected by abdominal massage was 1.2 ml. on the average. The collected fluid was pooled in a test tube at 0°C until the total amount reached 7—10 ml. As the protein concentration of the fluid was only 0.4 per cent, the fluid could not be analyzed in this state and it needed to be condensed. Then, the pooled fluid samples were condensed to 3 ml. by dialysis in 30 per cent arabic gum solution which was placed in a refrigerator at 4-6°C. It was more suitable to make the 30 per cent arabic gum solution with veronal buffer (0.05 M Sodium diethylbarbiturate, 0.01 M diethylbarbituric acid) rather than make it with distilled water. When the solution was made with water, some of the precipitation arose in the course of condensing although this precipitation was dissolved away by dialysis with a veronal buffer which was done before analysis; on the other hand, when a veronal buffer was used as solvent, none of the precipitation arose in the course of condensing. The protein concentration rose 1·-1.4 per cent in this manner. The concentrated fluid was placed in cellophane tubing and dialyzed against the veronal buffer mentioned above (pH 8.6, ionic strength 0.06) at 4·-8°C for a period of 1 or 2 days. Electrophoretic analysis was also performed with veronal buffer, employing the Hitachi Electrophoresis Apparatus with micro cell. Electrophoresis was carried out at five milliamperes and approximately 140 volts for about 3600 seconds. The relative mobilities of various components, which are the relative percentage of displacements of each peak compared with the displacement of component 1 as 100 per cent, were calculated from an electrophoretic diagram, and from these mobilities corresponding components of each diagram were identified. The relative per cent composition of the components was determined by the method of Longsworth (1942).

Blood samples were obtained from the brachial vein of the same bird from which was collected the transparent fluid samples. Just after collection, the blood sample was placed in an incubater at 38°C and when clotting appeared to be complete, the sample was placed into a refrigerator and allowed to stand for about 16 hours to separate the blood serum. The serum was dialyzed with a veronal buffer and analyzed electrophoretically.

The protein concentration of both serum and the transparent fluid was determined with the Hitachi protein refractometer and the fluid

protein concentration was again ascertained by the semi-micro Kjeldahl method. The concentration of the fluid was determined as 0.38 and 0.43 per cent on the average with the refractometer and Kjeldahl methods. On the other hand, serum had an average concentration of 5.1 per cent. The final protein concentrations of serum and the fluid were 2.0—3.0 per cent (on an average, 2.5 per cent) and 0.4—0.8 per cent (on an average, 0.6 per cent) respectively.

Results and discussion

The blood protein components of the fowl have been studied by Tiselius electrophoretic method or by the method of filterpaper electrophoresis (Sanders et al., 1944; Deutsh and Goodloe, 1945; Moore, 1945, 1948; Brandt et al., 1951; Clegg et al., 1951, 1953; Common et al., 1953; McKinley et al., 1953). In general, it has been noticed that the plasma of the fowl contains 6 components and serum contains 5 owing to the lack of fibrinogen. These fractions are designated usually as albumin, α_1 globulin, α_2 globulin, β globulin, fibrinogen and γ globulin, by analogy with human plasma fractions.

In the preliminary experiments, the writer photographed many plasma and serum patterns of the white Leghorn cocks which were analyzed in the boric acid buffer after Brandt et al. (1951), and plasma patterns were identical to those represented by Sanders et al. (Fig. 1 a).

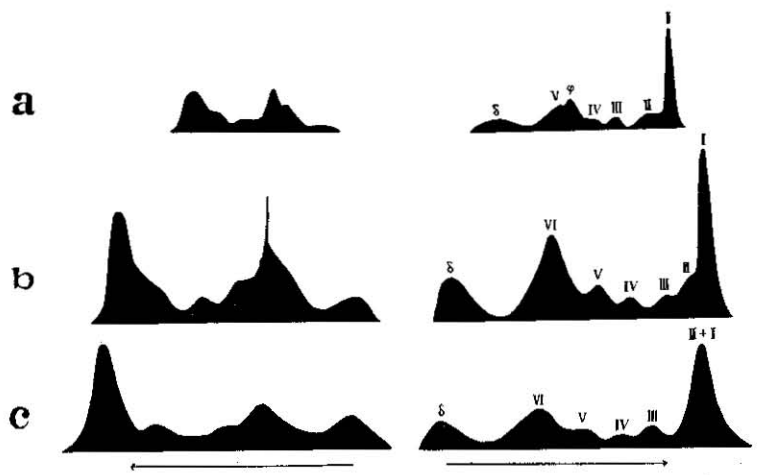

Fig. 1. Electrophoretic patterns of plasma, serum and transparent fluid of the cock. a. Plasma pattern analyzed with boric acid buffer. b. Serum pattern analyzed with veronal buffer. c. Pattern of transparent fluid analyzed with veronal buffer.

In the cock serum, it lacked a fibrinogen peak and there were 5 peaks. Component 1 to 5 in order of decreasing mobility was designated respectively as albumin, α_1, α_2, β and γ globulin, from the result of this experiment. On the other hand, in the patterns of the cock serum which were analyzed with veronal buffer as in this experiment, 6 peaks were observed in almost all cases, including a new peak situated between component one and two, in respect of their relative mobilities (Fig. 1 b, Table 1). McKinley et al. (1953) who studied the chicken serum by paper electrophoresis, demonstrated 6 components and they designated these fractions as albumin, α_1, α_2, α_3, β and γ globulin. Then, the fractions which had been considered as component 2 (α_1) and 3 (α_2) in the patterns which were analyzed with boric acid buffer it might be more suitable to designate as component 2 plus 3 ($\alpha_1+\alpha_2$) and 4 (α_3) respectively.

Table 1. Electrophoretic analyses of transparent fluid and blood serum in the cock.

	Relative per cent composition						Relative mobilities (%)					
	1	2	3	4	5	6	1	2	3	4	5	6
Transparent fluid	52.4	–	5.8	2.9	6.0	32.9	100	–	82	71	56	40
	50.4	–	8 6	3.3	6.3	31.4	100	–	82	69	60	37
	46.8	–	9.8	5.6	8.4	29.4	100	–	81	69	57	38
Blood serum	31.0	8.6	4.8	6.0	9.8	39.8	100	93	81	70	57	40
	33.4	7.3	5.0	5.9	8.9	39.5	100	90	83	71	58	42
	30.1	10.2	6.4	6.0	10.5	36.8	100	91	81	70	58	39
	27.0	8.6	6.9	5.6	11.1	40.8	100	93	83	69	57	38

The protein concentration of the transparent fluid was only 0.4 per cent as mentioned before and it was much lower than that of blood serum. However, this fact does not deny the assumption that the transparent fluid is a fluid similar to lymph. Because the protein content of lymph is less than blood serum and the concentration varies markedly in different regions of the body. Lymph coming from the liver has a relatively high protein content, about 5 per cent, lymph obtained from subcutaneous tissues, however, contains less than 1 per cent protein under normal conditions (Cantarow and Schepartz, 1954). Low protein concentration of the transparent fluid is presumably based on the facts that the fluid was generated from the lymphoid tissue or the vascular body which laid subcutaneous and that the amount of the fluid generated was very large in a short time (Cf. Nishiyama, 1955).

The relative per cent composition of the transparent fluid compared with blood serum is presented in Table 1 and Fig. 2, and the average value for each component is as follows: component 1 and 2, 50 per cent; component 3, 8 per cent; component 4, 4 per cent; component 5, 7 per cent and component 6, 31 per cent. Assuming the value of component 2 of the transparent fluid was the same as that of blood serum, i.e., 9 per cent, the relative per cent composition of the component 1 (albumin fraction) became 41 per cent. Still, the largest difference between fluid and serum was seen in component 1.

It is said that the relative portion of the protein of the lymph is almost the same as that of blood serum, although the albumin fraction of the lymph is somewhat larger in amount than blood serum (Hirai, 1953).

These evidences mentioned above support the assumption that the transparent fluid is a fluid similar to lymph.

In the previous paper (Nishiyama, 1955), the writer had presumed that the very small amount of secretion from the epithelial cells of the lymph-folds might be added to the lymph to make up the transparent fluid. The result of this experiment, however, revealed that the secretion to be added, if any, was negligible.

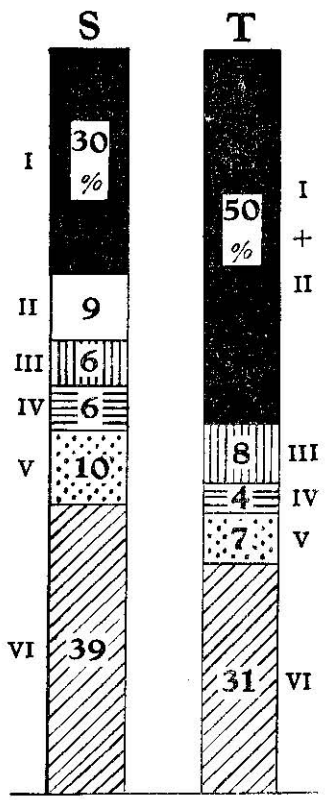

Fig. 2. Average value for each component of serum and transparent fluid.
S=Serum. T=Transparent fluid.

Summary

1. Transparent fluid, accessory reproductive fluid of cocks, contained all protein fractions which were contained in blood serum. The protein concentration of the fluid was, however, very low, i.e., 0.4 per cent on an average.

2. The relative per cent composition of the protein fractions of

the transparent fluid was similar to that of blood serum, although the albumin fraction of the fluid was larger than serum.

3. The results of this experiment support the assumption that the transparent fluid is a fluid similar to lymph.

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