《Research note》

An Immunological Method of Measuring Chicken Serum Vitellin

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The measurement of serum vitellin in chicken has been carried out using ring precipitin test¹⁾. HOMMA *et al.*²⁾ reported a method in which the serum vitellin was measured by turbidity of the serum treated with acetic acid buffer. TANABE *et al.*³⁾ revealed that serum vitellin developed in the sera of laying hens composed of two components with antigenicity. On the other hand, LAURELL⁴⁾ reported a simple immunological measurement, the electroimmunodiffusion, which was available for quantitative analysis of serum protein.

The purpose of this paper is to report a more precise method of measuring the serum vitellin in chicken by the use of the electroimmunodiffusion of LAURELL⁴⁾.

Methods and Materials

Anti-serum vitellin serum was prepared by immunizing rabbits with the pooled sera from 10 laying hens. The unabsorbed antiserum was repeatedly absorbed with cockerel serum to remove species-specific antibodies. Electroimmunodiffusion was carried out by the method described by LAURELL⁴⁾ with minor modification as follows. Pre-cleaned glasses of 8×8 cm in size were covered with the mixture consisting of 6 ml of 1% agarose and 0.4 ml of antiserum. After solidification, 9 holes in 2.6 mm in diameter were punched out along with a line 1.8 cm apart from an edge of the plate. The serum of 0.06 ml was put on the each hole. The electrophoresis was run under the constant current of 15 mA for each plate for two hours. The pooled serum from 10 normal laying hens was used as a standard laying hen serum. After the electrophoresis, the plate was turned over and a migration distance (height of the precipitation line) was measured with a rule under oblique illumination.

To investigate changes of serum vitellin concentration with growth of chicken, 28 White Leghorn chickens of 11 weeks of age were bled weekly until 24 weeks of age. Also, ultracentrifugation was performed on pooled serum to examine heterogeneity of vitellin. The sera obtained from laying hens, cockerels and growing female chickens were centrifuged at 40,000 rpm (100,200×g) at 5°C for 4 hours on a HITACHI ultracentrifuge. The sera were separated into three partitions according to TANABE *et al.*³⁾ and ABE *et al.*⁵⁾ Three partitions, top layer (L I), middle layer (L II) and bottom sediment (L III), were prepared from the serum of laying hens. Similarly, CO I, CO II and CO III

were prepared from the serum of cockerels and CH I, CH II and CH III from that of growing female chickens. The reaction patterns of these partitions were compared with that of whole serum of laying hen by electroimmunodiffusion and immunoelectrophoresis.

Results and discussion

An electroimmunodiffusion analysis of serial two-fold dilution of a standard serum of which dilutions up to 256-fold gave a positive reaction by ring precipitin test was shown in Fig. 1. Quantitative analysis on sera diluted less than two-fold was impossible, because tops of precipitation line became faintly round. However, within the range

Fig 1. Electroimmunodiffusion patterns of serial dilutions of a standard laying hen serum, developed with anti-serum vitellin.

Fig 2. Electroimmunodiffusion patterns of ultracentrifuged partitions of chicken serum.

L I, L II, L III: Ultracentrifuged partitions of laying hen serum.
CH I, CH III: Those of growing female chicken serum. CO I, CO II
CO III: Those of cockerel serum.

from 4 to 256-fold, lengths of precipitation lines were linearly reduced with dilution. The maximum dilution showing a positive reaction was 256-fold which accorded with the result of ring precipitin tests. A dark and short precipitation line was recognized inside of light and long one. Therefore, it was considered that serum of laying hen had two antigens which reacted with anti-serum vitellin as described by TANABE *et al.*⁸⁾ The regression lines of two precipitation lines were shown in following formulas $Y=2.52-0.07 X$ for the long precipitation line in range from 4 to 256-fold, $Y=2.17-0.14 X$ for

Fig 3. Immunoelectrophoresis of ultracentrifuged partitions of chicken serum, developed with anti-serum vitellin.
CH III, L I, L II, L III, CO III: See the Fig. 2.

Fig 4. Changes of serum vitellin concentration with growth of chicken.

the short precipitation line in range from 4 to 64-fold dilution, where Y is common logarithm of number of dilution, X is length (mm) of the precipitation line.

Electroimmunodiffusion analysis of ultracentrifuged partitions was shown in Fig. 2. The antigen reacting with anti-serum vitellin was not recognized in each partition of the cockerel and growing chicken sera. As to the partitions of laying hens, L I produced a dark and short precipitation line and L II and L III a long one. as shown in Fig. 3, immunoelectrophoretic analysis of the partitions represented the results which corresponded to those of electroimmunodiffusion; a long precipitation line was seen at L II and L III and a short one at L I. TANABE *et al.*⁹⁾ reported that L I consisted of a low density phospholipid-free protein and L II and L III a phosphoprotein or a phosphoprotein complex. Therefore, it might be probable that these components could be measured individually by this method.

In regard to the change of serum vitellin (the long precipitation line) with growth of chicken, no precipitation line was observed until 13 weeks of age. However, serum vitellin developed in 1 out of 28 birds at 14 weeks of age, 5 at 15 weeks, 13 at 16 weeks, 20 at 17 weeks and all birds until 22 weeks. The concentration of serum vitellin increased remarkably one or two weeks after the development of serum vitellin. The serum with such high concentration of serum vitellin could not accurately examined unless it was diluted appropriately, usually 50-fold. Changes of serum vitellin concentration of growing female chickens of which serum vitellin was first observed at 15, 16 and 17 weeks of age was shown in Fig. 4. On the chickens which detected serum vitellin at 15 weeks of age, the length of precipitation line of serum diluted 50-fold increased 4.7 mm after one week, and reached a peak (13.8 mm) after 3 weeks and then decreased. For the 23 birds which laid up to 24 weeks of age, relationship between age of serum vitellin occurrence and that of the first egg was investigated. The coefficient of correlation was 0.41.

From the present experiment, it was concluded that electroimmunodiffusion could be utilized as an immunological measuring method of serum vitellin of the hen.

Literature

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鶏血清ビテリンの免疫学的測定方法

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鶏血清ビテリンの免疫学的な測定方法として、従来から重層沈降反応が用いられてきたが、より正確な定量的方法として、LAURELLの電氣的免疫拡散法によって血清ビテリンの測定を行なった。

電氣的免疫拡散法は次の方法で行なった。1% アガロース液 6 ml に抗ビテリン血清 0.4 ml を混合し、8×8 cm の大きさのアガロースゲルプレートを作った。陽極側に試料を入れ、各プレート当り 15 mA で 2 時間泳動し、沈降線の長さから血清ビテリン量を求めた。

10 羽の産卵鶏の混合血清を標準試料として、2ⁿ 稀釈して泳動した結果、各試料とも長い沈降線とその内側の短い沈降線の 2 本が認められ、長い沈降線は 4~256 倍稀釈の範囲において、 $y=2.52-0.07x$ 、短い沈降線は 4~64 倍において、 $y=2.17-0.14x$ の直線回帰式が得られた (y は稀釈倍率の常用対数、 x は沈降線の長さ、mm)。したがって、この範囲のビテリン濃度においては、血清ビテリンの測定に電氣的免疫拡散法が利用できる。

發育に伴う血清ビテリン濃度を長い沈降線により測定した結果、14 週齢においては、28 羽中 1 羽に沈降線が認められ、22 週齢までにすべての鶏の血清中にビテリンが検出された。ビテリン濃度はいずれの場合も、最初に沈降線が認められた翌週に著るしく増加し、3~4 週間後に最高値となり、その後減少した。また、ビテリンの出現週齢と初産日齢の間の相関係数は 0.41 で有意な値であった。

産卵鶏血清を 100,200×g で 4 時間遠心し、上、中、下の 3 層に分画して、それらの分画と抗ビテリン血清との反応を電氣的免疫拡散法と免疫電気泳動で検討した。全血清において認められた長い沈降線は超遠心分画における中、下層に相当し、短い沈降線は上層と一致した。これらのことから、産卵鶏血清の上層分画に含まれる low density の磷脂質蛋白質と下層分画に含まれる磷脂蛋白質を、それぞれ別個に測定できる可能性が示唆された。

(家禽会誌, 13, 243~247, 1976)