Immunochemical Investigation of the Serum Proteins in Chick Embryos

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INTRODUCTION

The use of specific antisera against different embryonic antigens to detect a correlation between morphological and immunochemical differentiation in ontogenesis has proved to be a valuable technique.

In a study of the appearance of lens proteins in chick embryos, using various antisera and agar precipitation methods, we observed the development of precipitation lines which did not correspond to specific lens proteins. These lines were encountered in the Ouchterlony and immunoelectrophoretic pictures obtained with young (up to 120 hr.) embryo or lens extracts when these were tested with antiserum to 24-hr. embryo extract or to adult chick serum. However, this type of precipitation band was never found when antiserum against adult chick lens was used.

Some of our experimental results which clarify the nature of these precipitation reactions are summarized below. Full details of these experiments are the subject of another publication.

1. As the antiserum against adult chick serum formed several precipitation lines with young stage embryo extracts, the presence of serum proteins in these extracts was clearly demonstrated.

2. The antiserum against 24-hr. embryo extract formed five precipitation lines with the homologous antigen solution, three lines with adult chick serum and also five lines with defatted yolk. The antiserum was prepared by immunizing rabbits with a series of 24-hr. embryo extracts. The purpose of the latter procedure was to detect traces of lens antigen in early

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stages of development, i.e. to use specific antibody production in animals, which is a most sensitive method for the detection of minimal quantities of antigen.

3. However, no precipitation reaction occurred between anti-24 hr. embryo-extract serum and 0-02-5 per cent. (wet weight) adult chick lens extracts; one to two lines were obtained with a 120-hr. lens extract, which did not appear when the antiserum was absorbed with adult chick serum.

4. When the anti-24 hr. embryo-extract serum was well absorbed with yolk, precipitation lines with chick serum and 24-hr. embryo extract were no longer formed. After absorption of the antiserum with adult chick serum it formed only two precipitation lines with yolk and 24-hr. embryo extracts.

From these experiments we concluded that the 24-hr. embryo extract contains serum and yolk proteins and very probably no lens antigens. The occurrence of precipitation lines when 120-hr. lens extracts are tested with anti-chick serum and anti-24 hr. embryo extract, indicates the possible presence of serum antigens in 120-hr. embryo lenses. As the 24-hr. embryo extract forms no precipitation lines with antiserum against adult chick serum, after the antiserum has been absorbed by yolk proteins, we believe that the 24-hr. chick embryo possesses the same serum proteins as were demonstrated to be present in yolk. For this reason we fully support Nace's (1953) hypothesis that yolk is the source of the early serum proteins in the embryonic blood.

In a serological study on the origin of the later-appearing serum globulins in chick embryos, Schechtman & Hoffman (1952) thought they had found experimental evidence to support Sabin's hypothesis that embryonic serum originates from disintegrating blood cells at the 4th-6th day of development. They used antisera against the α- and β-globulin fractions of adult chick serum. However, these antisera were far from specific and showed considerable cross reactions with albumin, gamma globulin and defatted yolk. By means of the ring test the authors studied the precipitation reaction between dilutions of lyophilized serum or total blood extracts and the antisera, which were previously absorbed with albumin and/or gamma globulin. The absorption of the antisera with albumin and/or gamma globulin was meant to make the antisera more specific for the α- and β-fractions. After this procedure the antisera no longer gave a precipitation reaction with the antigens used for absorption, but were also weaker in antibody titre against the α-β globulin fractions. Schechtman and Hoffman concluded this from the fact that the absorbed antisera no longer precipitated small concentrations of the α-β globulins, but much higher concentrations. They found no precipitation between the absorbed antisera and undiluted or up to 1/1500 diluted 6th–21st day embryo serum, but did find a positive reaction with the 3rd–21st day total blood extracts in relatively high concentrations. Schechtman and Hoffman then stated: 'Weak antisera
thus prove to be of great advantage, for substances of $\alpha-\beta$ globulin antigenicity must be released in considerable amounts by cellular destruction if they can be detected by antisera too weak to react with serum from any stage of development.' Here we think Schechtman and Hoffman were wrong in the interpretation of the quantitative relation between antigen–antibody ratio and the resulting specific precipitate. It is well known from immunological precipitation curves that rabbit immune sera, when the antibody titre decreases, generally detect only smaller concentrations of the corresponding antigen.

How Schechtman and Hoffman came to their experimental results may no doubt be explained by the fact that the chick serum components were roughly fractionated, and thus were neither suitable test antigens nor pure enough for specific absorption of the antisera.

In regard to this problem, we performed some experiments to demonstrate the possible presence of serum proteins in 4th-day blood cell lysates. The reason why the 4th-day blood was chosen was that it is at about this time that cell disintegration starts. If present, the serum proteins should at least be demonstrated in the lysate of these primitive blood cells, which will undergo destruction the following 24–48 hr. of embryonic development.

**MATERIALS AND METHODS**

*Preparation of the blood cell lysate*

For each experiment:

(a) Whole blood was collected from thirty 4-day-old chick embryos. During the collection of the blood there occurred a contamination with yolk which caused agglutination of the blood cells. The blood cells were washed four to six times with an excess of physiological salt solution (1 vol. of cells to 20 vol. of 0.9 per cent. NaCl sol.) to remove adhering plasma proteins and yolk. After washing macroscopically perceptible agglutination of the cells existed no longer.

In experiment I, 0.15 ml. packed cells was added to 0.45 ml. and in the other experiments (II–VI) to 0.20 ml. of distilled water. After 2 hr. at 37°C. the suspension was kept at +4°C. for one night, or was frozen at –20°C. and subsequently thawed. In both ways a total lysis of the cells was obtained. The lysates (containing the stromata) were investigated on Ouchterlony plates.

(b) In the same way a lysate of adult chick blood cells was prepared. These blood cells were washed both after, and without, incubation in yolk.

*Antisera*

Two types of antisera were used. They were prepared in rabbits by means of hyperimmunization and the most potent immune serum was used in these experiments.
The antigen mixtures were mixed with complete Freund's adjuvant and injected intramuscularly.

(a) Antiserum against 24-hr. embryo extract. This antiserum contained three precipitating antibodies against adult chick serum and two other antibodies directed against specific yolk proteins (vide introduction).

(b) Antiserum against adult fowl serum. This antiserum was made in a rabbit which produced many different precipitating antibodies. Immunoelectrophoresis revealed more than ten precipitation lines which were located in all electrophoretic areas. This antiserum was used undiluted, and diluted 50 per cent. to change the location of the precipitation lines in the diffusion area, and thus facilitate the assessment of any immunological identity reaction.

**Technique**

The Ouchterlony technique was performed with cups containing 0.02 ml. antiserum and 0.02-0.2 ml. antigen solution. The distance between antigen and antiserum cups measured 5 mm.

**RESULTS AND DISCUSSION**

In the first experiment neither serum directed against adult chick serum nor anti-24 hr. embryo-extract serum precipitated with any dilution of the 4th-day lysate. We then concentrated the lysate by adding less distilled water to the blood cells for lysis (experiment II) and observed one precipitation line, formed by each of the two antisera. The lines were too short for the possible recognition of an identity reaction.

We wondered if the presence of yolk in the 4th-day blood might cause contamination of the cell surface which, due to clumping of the cells, could not be removed properly by washing. If that were true, the incubation of adult chick blood cells in yolk should give the same result, whereas the lysate of adult chick blood cells, which had not been incubated in yolk, should not be demonstrated to contain a serum protein. This, indeed, appeared to be the case (experiment III).

We assumed, when serum contamination of the blood cells' surface was engaged, prolonged washing might be useful. Instead of four times, now the cells were washed six times and special attention was paid to the removal of all little clumps of yolk.

From the experiments IV–VI it can be seen that no serum protein was precipitated, either with 4th-day lysate or adult chick blood cell lysate, after previous incubation in yolk.

The precipitation tests, performed by Boyd's ring test, gave the same results.

It thus appears impossible to demonstrate the presence of serum proteins in properly washed blood cells collected from chick embryos on the 4th day of development. In the supernatant serum of these blood cells several proteins
Experimental results

Experiments I–III, washed four times
Experiments IV–VI, washed six times

<table>
<thead>
<tr>
<th>Antisera</th>
<th>Anti-24 hr. embryo extract serum; precipitation reaction on Ouchterlony plate</th>
<th>Undiluted or 50 per-cent diluted anti-adult chick serum; precipitation reaction on Ouchterlony plate</th>
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<tbody>
<tr>
<td>I 4th-day lysate</td>
<td>0·20 ml.</td>
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<td>0·10 ml.</td>
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<td></td>
<td>0·016 ml.</td>
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<td>II 4th-day lysate</td>
<td>0·20 ml.</td>
<td>One line</td>
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<tr>
<td>III (a) 4th-day lysate</td>
<td>0·20 ml.</td>
<td>One line</td>
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<tr>
<td>(b) adult chick blood cell lysate without incubation in yolk</td>
<td>0·20 ml.</td>
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<tr>
<td>(c) adult chick blood cell lysate; previous incubation of the blood cells in yolk</td>
<td>0·20 ml.</td>
<td>One line</td>
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<tr>
<td>IV (a) 4th day lysate</td>
<td>0·20 ml.</td>
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</tr>
<tr>
<td>(b) adult chick blood cell lysate after incubation of the blood cells in yolk</td>
<td>0·20 ml.</td>
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<td>V and VI as in IV (a) and IV (b)</td>
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were found to be present. However, these proteins belong also, in addition to the serum, to the contaminating yolk. In our opinion one should not accept a causal relationship between the disintegration of primitive blood cells and the appearance of α- and β-globulins in the blood. It seems much more likely that during the 4th to 6th day of development, cell differentiation and maturation lead to the onset of functioning of both blood cells and serum protein-producing tissues.

SUMMARY

In this paper, experiments which were performed to investigate the possible presence of serum proteins in blood cells of young stage chick embryos are
described. From the results we conclude that serum proteins are not present in primitive blood cells at a time shortly before their disintegration. Our findings are not in accordance with those of Schechtman & Hoffman (1952) who postulated that the appearance of $\alpha$- and $\beta$-globulins in the blood-stream is a result of the liberation of these globulins during the destruction of primitive blood cells. We are of the opinion that the maturation of blood cell-forming tissue, which results in the formation of a new blood cell population, goes parallel or coincides with the functional development of protein-forming tissues.

RÉSUMÉ

Recherches immunochimiques des protéines du serum d'embryons de poulet

Les expériences décrites dans le présent travail ont pour objet de rechercher la présence possible de protéines sériques dans les cellules sanguines d'embryons de poulet de jeunes stades. D'après nos résultats, nous concluons que les protéines sériques ne sont pas présentes dans les cellules sanguines primitives juste avant leur destruction. Nos résultats ne concordent pas avec ceux de Schechtman et Hoffman (1952) qui postulent que l'apparition des $\alpha$ et $\beta$-globulines dans le courant sanguin résulte de la libération de ces globulines au cours de la destruction des cellules sanguines primitives. Nous pensons que la maturation des tissus hématopoïétiques, qui aboutit à la formation d'une nouvelle population de cellules sanguines, va de pair ou coïncide avec le développement fonctionnel de tissus formateurs de protéines.

REFERENCES


(Manuscript received 14th December 1962: revised 29th April 1963)