Transient embryonic antigens in the chick

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WITH TWO PLATES

INTRODUCTION

The presence of an organ antigen in the early chick embryo was first demonstrated by Schechtman (1948). He found that an antigenic substance common to brain, heart, liver and muscle of chicks at hatching is already present in primitive streak and early neurula stages of the embryo. This observation, with respect to brain and heart, was subsequently confirmed by Ebert (1950). McCallion & Langman (1964) have recently demonstrated that there are at least eight antigenic substances in the adult chicken brain that are class-specific but that are more or less common to other organs, with only quantitative differences. These authors have further demonstrated that there are at least three, possibly as many as five, antigenic substances in adult chicken brain that are not only class-specific but also tissue-specific, occurring only in the brain, spinal cord, nervous retina and nerves. The non-specific antigens appear progressively during the first 4 days of incubation. The first of the brain-specific antigens appears at the end of the 5th day of incubation and all are present by the end of the 12th day of incubation, that is, during the establishment of the definitive cytoarchitecture of the brain.

No evidence has yet been presented that would suggest that any tissue-specific adult antigens occur in embryonic neural tissue during the formation of the neural plate, closure of the neural tube and the establishment of the primitive brain vesicles. On the assumption that there might be one or more transient embryonic antigens related to these stages of neural development, antisera against 9-day embryonic brain have been prepared in order to determine, by means of the double-diffusion technique of Ouchterlony (1953), whether any saline-soluble antigens are present in the early embryo that are not found in the adult.

MATERIALS AND METHODS

Preparation of antisera

Brains were removed from chick embryos on the 9th day of incubation and freed of all other tissues and the eyes. The brains were homogenized in a small

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amount of 0·9 per cent. saline solution and allowed to extract at 4°C. for 3–4 hr. The homogenate was centrifuged at 20,000 Xg for 20 min. After centrifugation the supernatant was suspended in an equal volume of Freund's adjuvant (Difco Bacto-adjuvant) and 1 ml. of the suspension was injected subcutaneously into rabbits at four different sites. After an interval of 30 days the rabbits were again injected and for a third time after an interval of 7 days. Good antisera were obtained after a lapse of 10 days.

Preparation of tissue extracts

Saline extracts of adult brain tissue and of embryo brain from 3 days until hatching were prepared as described above and used in agar-diffusion tests. Similarly, saline extracts of several 9-day embryo organs and of adult organs were prepared for agar-diffusion tests.

Agar-diffusion technique

Agar plates were prepared as previously described by Langman (1959) and by McCallion & Langman (1964) with 2 per cent. dialysed filtered agar (Difco; B 140; pH 7·2) to which 0·01 per cent. merthiolate had been added. The antiserum was placed in a central well in the agar plate and the test extracts were placed in peripheral wells, at a distance of 13 mm. from the central well. The tests were carried out at 37°C. and the plates were read after 7 days. Subsequently the agar plates were dialysed against saline solution and photographed.

RESULTS

9-day embryo brain antigens

When saline extract of 9-day chick embryo brain was tested against 9-day brain antiserum by means of the agar-diffusion test a precipitin band was formed between the two wells near the antigen well. This band was also formed with serum and is hereafter referred to as the serum band. In addition to a number of common tissue precipitin bands two significant groups of precipitin bands were formed. One group of strong bands was formed near the antiserum well (Plate 1, Fig. 1). This group consisted of at least three bands which coalesced as a single precipitin line in some plates, was resolved in others into two bands and could be shown to be composed of three bands when compared with brain extract of older embryos (Plate 1, Figs. 1 and 4). A group of two rather weak precipitin bands was formed between the serum band and the strong bands (Plate 1, Fig. 1).

Adult brain antigens

The precipitin pattern obtained between saline extracts of adult brain and 9-day brain antiserum was composed of only four bands. The serum band was formed.
Photographs of agar-diffusion plates showing the precipitin lines formed between 9-day chick brain antiserum and various embryonic and adult tissue extracts. Fusion of precipitin lines formed by extracts in adjacent wells indicates identity of the antigenic component of the extracts. Antiserum in central well in all figures. Key to extracts tested: S = serum; 9 = 9-day brain; B = adult brain; L = adult liver; K = adult kidney; H = adult heart; 9St = 9-day stomach; 9Lu = 9-day lung; 9Sk = 9-day skin; 9M = 9-day muscle; 9L = 9-day liver; 9K = 9-day kidney; 9H = 9-day heart; numerals = age of embryonic brain in days; s = serum band; w = weak bands; st = strong bands.

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PLATE 2

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Of the strong bands only two were formed (Plate 1, Fig. 2) and of the two weak bands only one was formed (Plate 1, Fig. 2).

Brain antigens in the course of development

Saline extracts of the brain of the chick embryo, from 3 days of incubation until hatching, were tested against the 9-day brain antiserum. The serum band was formed even by the earliest extract tested. The group of three strong bands was formed with extracts of brain by the 3rd day of incubation (Plate 1, Fig. 3). The strong bands are sometimes coalesced and at other times are resolved as distinct bands. Therefore, two of them are apparent and the third is probably also present. By the 19th day the antigen responsible for one of these bands has begun to disappear from the brain and has disappeared at hatching (Plate 1, Fig. 4). The other two remain even in the adult brain. On the 5th to the 6th day of incubation one of the weak bands is formed between the wells (Plate 1, Fig. 3) and the second weak band is formed about the 8th day of incubation (Plate 2, Fig. 5). Between the 12th and the 13th day of incubation the antigen responsible for one of the weak bands increases in concentration (Plate 2, Fig. 6), decreases again about the 15th day, and disappears before hatching (Plate 1, Fig. 4).

Comparison with adult chicken organs

Of the three strong precipitin bands formed between 9-day brain extract and 9-day brain antiserum only two are formed between adult brain extract and the antiserum (Plate 1, Fig. 1). At least one of them is also formed with adult liver extract and heart extract (Plate 1, Fig. 1) but neither is formed with adult serum (Plate 1, Fig. 1; Plate 2, Fig. 6). Of the two weak bands only one is weakly formed with adult brain (Plate 1, Fig. 1) and the same one seems to be formed with liver extract but not with heart extract or serum (Plate 1, Fig. 1, Plate 2, Fig. 6). The serum band was formed with all organs tested.

Comparison with 9-day chick organs

All of the strong precipitin bands were formed between the 9-day brain antiserum and extracts of every 9-day organ tested (brain, liver, heart, kidney and muscle) (Plate 2, Fig. 7, 8). The antigen of one of the strong bands disappears from these organs at about the time of hatching (Plate 2, Fig. 7). At least one of the two weak precipitin bands is specific for 9-day brain and is not formed with other 9-day organs. Only the inner one of these bands is formed with brain after hatching (Plate 1, Fig. 4; Plate 2, Fig. 7).

DISCUSSION

In the field of immunoembryology antibodies directed against adult antigens have been used to study the synthesis and localization of antigenic substances during the course of development. In many cases it has been possible to relate
such substances to visible morphogenetic events (Ebert, 1959). Adult organ antisera have been used to analyse the development of the adult spectrum of antigenic substances of the lens (Maisel & Langman, 1961; Maisel & Harmison, 1963), of the liver and kidney (Croisille, 1960; D'Amelio et al., 1963; Okada & Sato, 1963) and of the central nervous system (McCallion & Langman, 1964).

In this way McCallion and Langman have characterized the antigenic pattern of the adult chicken brain. These authors have demonstrated a group of at least three class-specific and tissue-specific antigens in the adult brain. These antigens appear progressively, one by one, in the developing brain of the chick embryo from the 5th to the 12th day of incubation and the adult pattern is then established. There was no indication from this study of the appearance of brain-specific adult antigens during earlier stages of the development of the neural tube. The method could not, of course, discover any transient embryonic antigens.

Since embryonic tissues may possess antigenic substances no longer present in fully differentiated adult tissues, embryonic tissues should be used to make antibodies for the detection of transient antigens (Ebert, 1959). Burke et al. (1944) did prepare antiserum against the brain of the 13-day chick embryo in order to study the antigenicity of the developing brain. Since the adult complement of brain-specific antigens is already present at 13 days of incubation (McCallion & Langman, 1964) it would appear to be more useful to obtain antisera against an earlier embryonic stage. In the present study it was felt that 9 days of incubation was the youngest stage from which a sufficient amount of tissue extract to promote antibody formation in the rabbit could be obtained from a reasonable number of embryos.

The results of Burke et al. (1944) suggest only a quantitatively specific brain antigen more or less common to other organs. Their 13-day brain antiserum, in precipitin tests, produced cross reactions with extracts of gonads, kidney and liver of 13-day embryos. Having reduced the cross reactions by absorption of the antiserum with extracts of these organs, these authors suggested that the 13-day embryonic brain contains an antigen common to the adult brain. In a study of the effects of brain antisera on explanted neural tissues Grunwalt (1949) found one alcohol-soluble antigen that was neural-specific and like that described by Burke et al. The latter authors described another antigen common to the 7-day brain but not to the adult brain. They obtained a precipitin reaction first with the 7-day brain. The reaction increased in intensity up to 13 days of incubation. They felt that their results clearly indicated a change in antigenicity in the developing brain.

In the present study the more sensitive technique of Ouchterlony (1943) was used with an antiserum against a younger embryonic brain. The results indicate that at least one and probably two neural-specific antigens are present in the brain of the chick embryo at 9 days of incubation, only one of which is still present in the adult brain. One of these antigens is present in the brain by the 6th day of incubation and the second is present by the 8th day. The latter is the one
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that disappears just before hatching and is a neural-specific transient antigen. It is probable that the antigen that persists in the adult brain is the first to appear of the three adult antigens described by McCallion & Langman (1964), but not comparable with the alcohol-soluble antigen of Grunwalt (1949). However, there is some doubt that this antigen is strictly neural-specific (compare L in Fig. 2 with L in Fig. 4). If this antigen is only quantitatively specific it could then be the same antigen as that described by Burke et al. (1944) and the alcohol-insoluble antigen of Grunwalt (1949).

In addition, one other antigen, which formed a strong precipitin band with the 9-day brain antiserum, is a transient embryonic antigen which disappears from the brain before hatching. This antigen, however, is common to several other embryonic organs. It is more likely that this antigen, rather than the one described above, is the same embryonic antigen detected by Burke et al. (1944).

It is evident from these results that there is at least one transient neural-specific antigen present in the developing brain of the chick embryo from the 8th day of incubation to near hatching. There is also one transient common organ antigen present in the embryo from at least the 2nd day of incubation to hatching. There is no evidence of a neural-specific antigen in earlier states of brain formation. There is certainly a change in antigenicity in the developing brain.

Lee et al. (1961), in a chromatographic study of soluble proteins extracted from chick brain at various stages of development, found that the protein pattern changes as the tissue matures. Whether this was due to the appearance or disappearance of some particular components or changes in relative amounts of the components during development was not revealed in their study. The results of the present study clearly suggest the disappearance of some components from the chick brain during the course of development.

SUMMARY

1. Immunochemical analysis of the 9-day chick brain with 9-day brain antiserum reveals significant amounts of two different groups of antigens. One group consists of at least three antigens. The other group consists of at least two antigens.

2. Comparison of 9-day brain with other 9-day organs and with adult organs, including adult brain, reveals that the group of three antigens are common antigens and that one of them is transient and disappears by hatching. It is further shown that of the group of two antigens at least one is neural-specific and transient and disappears from the brain by hatching.

RÉSUMÉ

Antigènes embryonnaires transitoires chez le poulet

1. L’analyse immunochimique de cerveau d’embryon de poulet de 9 jours avec du sérum anti-cerveau-de-9-jours révèle des quantités significatives de deux
groupes différents d’antigènes. L’un des groupes consiste en trois antigènes au moins. L’autre groupe consiste en deux antigènes au moins.

2. La comparaison de cerveau de 9 jours avec d’autres organes de 9 jours et avec des organes adultes, y compris du cerveau, révèle que le groupe de trois antigènes est constitué d’antigènes communs et que l’un d’eux est transitoire et disparaît à l’éclosion. On montre de plus que dans le groupe de deux antigènes, l’un au moins est spécifique du tissu nerveux et transitoire, et disparaît du cerveau à l’éclosion.

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REFERENCES


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