An immunological study on the effect of brain extract on the developing nervous tissue in the chick embryo

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WITH ONE PLATE

INTRODUCTION

In recent experiments 24–32 hr. chick embryos were treated with saline extract of adult chicken brain, which was injected into the yolk, into the sub-blastodermal space, or deposited over the blastoderm. After an additional 60-hr. incubation period, 30 to 40 per cent of the surviving embryos showed defects of the brain, spinal cord and eye, such as anencephalus, microcephalus, abnormal shape of the brain vesicles, rachischisis, anophthalmia and microphthalmia (Lenicque, 1959; Clarke & McCallion, 1959; Braverman, 1961). In addition, a number of embryos showed abnormal proliferation in the walls of the brain vesicles.

When other tissue extracts were examined it was found that the above-mentioned abnormalities could be produced only by saline extracts of chick brain and nervous retina, and not by extracts prepared from liver, spleen and skeletal muscle. The latter extracts do sometimes affect brain development, but then always in association with defects in other organ systems. Extracts prepared from brain tissue of the mouse and rat were likewise ineffective, when deposited over the chick blastoderm (Clarke & McCallion, 1959; Langman, unpublished). When brain extracts prepared from embryos of increasing age were tested, it was found that the component responsible for the abnormalities arises between the 6th and 12th day of embryonic development.

In an attempt to isolate the fraction responsible for the abnormalities, Lenicque separated the various components of adult chick brain extract by means of continuous flow electrophoresis and applied the fractions thus obtained to the embryo. The most negatively charged fraction was found to be the only component capable of producing abnormalities; the other fractions, however, were ineffective (Lenicque, 1959).

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Though the results obtained by the various workers are in general agreement, no satisfactory explanation has been offered as to the ‘specific inhibitory effect’ of homologous tissue homogenates on the development of the corresponding organ in the embryo, nor to the nature of the substance involved. Since it is likely that the effective brain component is a saline soluble protein, it was thought that the application of immunological techniques might give valuable information about the nature and number of the soluble components of brain extract. Moreover, as the effective component seems to arise between the 6th and 12th day of development, it was expected that immunological analysis of embryonic brain extracts might reveal the component responsible for the damaging effect on the embryo. In addition, comparison of the soluble antigenic components of the chick brain with those of the brains of other species and other chick organs would make it possible to explain why extracts of the brains of other animals and other chick organs did not produce any ‘inhibitory effect’ on the development of the chick brain.

Hence, in this work, adult chicken brain homogenate was analysed by means of the agar diffusion technique of Ouchterlony (1953), while in addition the appearance of the soluble components of the chick brain during development was studied. The data thus obtained were compared with those of other chick organs and with those of the brain of representative species of the vertebrate series.

**MATERIAL AND METHODS**

*Antibody preparation*

Brain tissue of adult chicken was removed from the crania, freed of meninges and blood vessels, and subsequently homogenized in small amounts of 0.9 per cent. saline. After centrifugation the supernatant was suspended in an equal volume of Freund's adjuvant (Difco Bacto-adjuvant), and 1 ml. of the suspension injected subcutaneously into a number of rabbits at four widely separated sites. This procedure was repeated at weekly intervals for several months. The antisera obtained from the various rabbits are referred to as brain antisera.

*Preparation of tissue extracts*

Brain tissue of adult chickens was dissected as described above and homogenized in 0.9 per cent. saline to a concentration of 250 mg. wet weight/ml. After centrifugation at 5000–10,000 r.p.m., the supernatant was collected and used for the agar-diffusion tests. Similarly, brain tissue obtained from embryos at various stages of development was homogenized in small amounts of saline and tested against brain antiserum. In the 24-hr. embryos, the entire area pellucida was dissected, washed free of yolk and used to prepare an homogenate.

Saline extracts of spinal cord, peripheral nerves, nervous retina, pigment retina,
PLATE

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iris, lens, skin, skeletal and cardiac muscle, liver and kidney of adult chicken were prepared in a manner as described for the brain. Before the tissues were removed the animal was perfused with saline. Brain extracts of various mammals, birds, reptiles, amphibians and fish were prepared in a manner similar to that of the chick.

Agar-diffusion technique

Agar plates were prepared as previously described (Langman, 1959), with 2 per cent. dialysed filtered agar (Difco; B 140; ph 7.2) to which 0.01 per cent. merthiolate had been added. Peripheral wells were made at a distance of 6–20 mm. from the central well and the tests were carried out at 37°C.

Explanation of Plate

Photographs of agar plates showing the precipitin lines formed by testing various tissue extracts against chick brain antiserum. Coalescence of precipitin lines formed by extracts in two adjacent wells indicates identity of the antigenic components; partial coalescence of precipitin lines indicates that the antigens have some reactive groups in common, but differ in others.

Figs. 1 and 2. Precipitin pattern formed by testing adult chicken brain extract against two different brain antisera. The top well contains chicken brain extract; the bottom well brain antiserum. Two groups of precipitin bands can be distinguished: an 'outer' group, marked 'o', which is located close to the antigen well, and an 'inner' group, marked 'i', located close to the antiserum well. Note the difference in reaction between the two antisera.

Fig. 3. Left top well contains adult chicken brain extract; right top well contains brain extract prepared from 60-hr. embryos; central well contains brain antiserum. The precipitin bands formed by the 60-hr. brain extract fuse with the 'inner' bands of the adult precipitin pattern, but not with the 'outer' bands.

Fig. 4. Left top well contains an extract of 24-hr. chick embryos, centre top well contains adult chick brain extract; right top well is filled with brain extract prepared from 9-day old chick embryos. Right bottom well contains brain extract of 6-day old chick embryos; middle bottom well contains 24-hr. chick embryo extract and left bottom well is filled with saline extract of yolk. The central well contains chick brain antiserum.

Note the fusion between the inner and outer precipitin bands formed by adult and 9-day brain extracts. The 6-day embryo brain extract forms a number of bands fusing with the 'inner' bands of the 3-day brain extract and one vague band which fuses with an 'outer' band. Extract of 24-hr. embryos forms two bands which fuse with the 'inner' bands of the adult brain extract, but fails to show any of the 'outer' bands.

Fig. 5. Left top well and right bottom well contain adult chick brain extract; right top well contains an extract of the chick nervous retina; right well, left bottom well and left well contain extract of iris, pigment retina and lens respectively. The 'outer' bands formed by adult chick brain extract are present in nervous retina extract, but not in extracts of iris, pigment retina and lens.

Figs. 6 and 7. Left top well contains an extract of chick skeletal muscle; centre top well and right top well contain an extract of adult chicken brain and chick serum respectively. Bottom well contains chick brain antiserum. The precipitin bands formed by chick muscle fuse with the 'inner' bands of chick brain extract. In Fig. 6 the chick serum proteins form a sharp band which seems to fuse with an 'inner' band; in Fig. 7, however, this band seems to fuse with one of the 'outer' bands of the brain extract.
Continuous flow electrophoresis

Twenty millilitres of fresh adult chicken brain was suspended in 80 ml. of 0.9 per cent. saline. The suspension was then centrifuged and run in a Spinco Model C.P. continuous flow electrophoresis apparatus for 36 hr. at 4°C. in Veronal buffer at pH 8.6 and ionic strength 0.02. The current used was 60 mA. The sample flow was adjusted to a rate of 3.2 ml./hr. The fractions collected were concentrated by forced evaporation through cellophane bags and subsequently tested against brain antiserum in the agar plate.

RESULTS

Adult brain antigens

When 10 per cent. saline extract of adult chicken brain was tested against chicken brain antiserum by means of the agar diffusion test, five to eleven more or less distinct precipitin bands were formed between the two wells (Plate, 1, 2). Further study showed that the bands were arranged in two distinct groups: one group composed of four to eight rather vaguely defined bands located close to the antiserum well, and a second group of one to three bands close to the antigen well. These two groups of precipitin bands are referred to as ‘inner’ and ‘outer’ bands respectively.

Brain antigens in the course of development

Table 1 shows the results obtained by testing extracts of 24-hr. embryos and brain tissue of embryos up to the 12th day of development against brain antiserum. It may be noticed that the precipitin bands formed by extracts of 24-hr. embryos and extracts of brain tissue from 36- to 96-hr. embryos gradually increase in number from one to six. With one particular antiserum this number increased to eight. When compared with the precipitin bands formed by adult brain extract, it is evident that those formed by extracts of embryo brain fuse with the ‘inner’ bands (Plate, 3, 4).

The first of the ‘outer’ bands becomes visible at the end of the 5th and beginning of the 6th day of development. The appearance of this band is followed by that of the other ‘outer’ bands, until by the 12th day of development, all are present.

Comparison with other chick organs

The precipitin patterns obtained by testing saline extracts of the various tissues of the adult chicken against brain antiserum were compared with that of adult chicken brain and the results are summarized in Table 2.

It was found that all tissue extracts, except the yolk of incubated eggs, gave rise to a number of bands which partially or completely fused with the ‘inner’ bands
### TABLE 1

*The appearance of precipitin bands in the course of brain development*

<table>
<thead>
<tr>
<th>Hours of incubation</th>
<th>Inner bands</th>
<th>Outer bands</th>
<th>Comparison adult and embryonic precipitin pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1–3</td>
<td>—</td>
<td><img src="image1" alt="Diagram" /></td>
</tr>
<tr>
<td>36</td>
<td>3–4</td>
<td>—</td>
<td><img src="image2" alt="Diagram" /></td>
</tr>
<tr>
<td>48</td>
<td>4–5</td>
<td>—</td>
<td><img src="image3" alt="Diagram" /></td>
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<tr>
<td>96</td>
<td>4–7</td>
<td>—</td>
<td><img src="image4" alt="Diagram" /></td>
</tr>
<tr>
<td>120</td>
<td>4–7</td>
<td>1</td>
<td><img src="image5" alt="Diagram" /></td>
</tr>
<tr>
<td>216</td>
<td>4–7</td>
<td>2</td>
<td><img src="image6" alt="Diagram" /></td>
</tr>
<tr>
<td>288</td>
<td>4–7</td>
<td>2–3</td>
<td><img src="image7" alt="Diagram" /></td>
</tr>
</tbody>
</table>

A—antigen adult, E—antigen embryonic, S—antiserum

### TABLE 2

*Precipitin bands formed by testing various chicken tissue extracts against chicken brain antiserum*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Inner bands</th>
<th>Outer bands</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ectodermal tissues</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>4–8</td>
<td>1–3</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>4–5</td>
<td>2–3</td>
</tr>
<tr>
<td>Peripheral nerves</td>
<td>4–5</td>
<td>2–3</td>
</tr>
<tr>
<td>Nervous retina</td>
<td>4–8</td>
<td>2–3</td>
</tr>
<tr>
<td>Pigment retina</td>
<td>4–8</td>
<td>—</td>
</tr>
<tr>
<td>Skin</td>
<td>4–6</td>
<td>—</td>
</tr>
<tr>
<td>Lens</td>
<td>4–8</td>
<td>—</td>
</tr>
<tr>
<td>Iris</td>
<td>4–8</td>
<td>—</td>
</tr>
<tr>
<td><strong>Mesodermal tissues</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>4–8</td>
<td>—</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>4–8</td>
<td>—</td>
</tr>
<tr>
<td>Kidney</td>
<td>4–8</td>
<td>—</td>
</tr>
<tr>
<td>Serum</td>
<td>2–4</td>
<td>?</td>
</tr>
<tr>
<td>Yolk</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Entodermal tissues</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>4–8</td>
<td>—</td>
</tr>
<tr>
<td>Spleen</td>
<td>4–8</td>
<td>—</td>
</tr>
</tbody>
</table>
formed by adult chicken brain extract. Only extracts of the nervous retina (Plate, 5), spinal cord and peripheral nerves gave rise to precipitin lines, which coalesced with the ‘outer’ bands. When the chick serum proteins were tested against brain antiserum, a number of bands became visible. Some of these fused with the ‘inner’ bands of brain extract but one rather sharp band sometimes seemed to fuse with one of the ‘outer’ bands, and sometimes with one of the ‘inner’ bands (Plate, 6, 7). This was the only case in which there was doubt whether a precipitin line was identical to an ‘inner’ or ‘outer’ band formed by brain extract.

Comparison with other brain extracts

Table 3 shows the results obtained by testing brain extracts of turkey, duck, mouse, rat, guinea pig, man, alligator, snake, frog and codfish against chick brain antiserum. While brain extracts of the turkey and the duck formed four to eight precipitin bands fusing with the ‘inner’, and one to three bands coalescing with the ‘outer’ bands formed by chick brain extract, that of the alligator formed one band fusing with the ‘inner’, and one or two bands fusing with the ‘outer’ bands of the chick brain precipitin pattern. Brain extracts of man, guinea pig, mouse, rat, snake and frog failed to form any precipitin bands when tested against chick brain antiserum.

**Table 3**

*Precipitin bands formed by testing brain extracts of various vertebrates against chick brain antiserum*

<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
<th>Inner bands</th>
<th>Outer bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammals</td>
<td>Man</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Guinea pig</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Birds</td>
<td>Chicken</td>
<td>4–8</td>
<td>1–3</td>
</tr>
<tr>
<td></td>
<td>Turkey</td>
<td>4–8</td>
<td>1–3</td>
</tr>
<tr>
<td></td>
<td>Duck</td>
<td>4–8</td>
<td>1–3</td>
</tr>
<tr>
<td>Reptiles</td>
<td>Alligator</td>
<td>1</td>
<td>1–2</td>
</tr>
<tr>
<td></td>
<td>Snake</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Amphibian</td>
<td>Frog</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fishes</td>
<td>Codfish</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Isolation of the brain antigens

When the various brain fractions, isolated by running adult brain extract in the continuous flow electrophoresis, were tested against brain antiserum, it was
found that the 'outer' bands were caused by those fractions which in the electrical field had moved towards the positive pole. The fractions which had moved towards the negative pole fused with the 'inner' bands formed by adult chick brain extract, while those in the middle field fused partly with the 'outer' and partly with the 'inner' bands.

**DISCUSSION**

The double diffusion test of Ouchterlony (1949, 1953) is based on the fact that antigens, moving away from the antigen well into the surrounding agar, are met by antibodies which migrate from the central well. When critical concentrations of an antigen and its corresponding antibody meet, a precipitin band is formed. Neither antigen nor antibody can diffuse beyond this precipitin zone, which acts as a virtual barrier to this particular antigen–antibody system. Other antigens and antibodies, however, pass through this precipitin line without being hampered in their migration (Wilson & Pringle, 1955; Korngold, 1956). When a mixture of several antigens and antibodies is used as in our experiments, a precipitin spectrum may be seen, each band corresponding to one antigen–antibody pair. Coalescence or fusion of precipitin bands, observed when various extracts are tested side by side in one agar plate, indicates identity of the antigens causing the formation of the bands.

Since in our experiments adult chicken brain extract gives rise to five to eleven precipitin bands when tested against chick brain antiserum, it is evident that the brain extract contains substantial amounts of at least five to eleven saline soluble components. This observation is in agreement with the work of LeBaron (1959), Kiyota (1959), Robertson (1960), and Bailey & Heald (1961a, b), who by means of paperstrip and starch gel electrophoresis demonstrated the presence of seven to fifteen soluble protein components in the vertebrate brain.

It is apparent from our observations that the antigenic brain components can be divided into two groups immunologically: one group which in the agar diffusion tests causes a number of bands close to the antibody well ('inner' bands) and a group which produces one to three precipitin bands close to the antigen well ('outer' bands). This more or less arbitrary division of the bands seems to be of essential importance since extracts of heart, skeletal muscle, spleen, liver, lens, iris and pigment retina, when tested against brain antiserum, formed precipitin bands which fused with the 'inner' bands only, and not with the 'outer' bands. This seems to indicate that the brain antigens responsible for the formation of the 'inner' bands are common organ antigens and have little or no tissue specific characteristics. The antigens causing the 'outer' bands, however, seem to be specific, since only nervous retina, spinal cord, and peripheral nerves contain antigens identical to these components.

When Clarke & McCallion (1959) and Lenicque (1959) examined the influence of adult brain homogenate on chick development, it was noted that the abnormalities were not restricted to the brain, but occurred in the eye as well. Likewise
when Lenicque (1959) and Langman (unpublished) tested the influence of total eye and nervous retina extracts, abnormalities were produced both in the eye and the brain of the embryos. On the contrary, extracts of skeletal and heart muscle, liver, kidney, lens, skin and brain of mammals did not cause specific malformations in the brain and the eye of the chick embryo. Hence, these experiments suggest that the components responsible for the specific neural and eye defects in the embryos are present in extracts of brain and nervous retina, but not in any other tissue extracts. Since brain and nervous retina have those antigens in common which in the agar diffusion test produce the ‘outer’ bands, and since none of the other tissue extracts possess these antigenic components, it is likely that these components are the cause of the abnormalities.

This possibility is supported by the results of experiments of Lenicque (1959), in which it was found that brain extract prepared from embryos younger than 6 days had no effect on embryonic development, while those from embryos older than 12 days had an effect comparable to that of adult brain extract. In view of our results this is not surprising. When brain extracts of embryos of increasing ages were tested against brain antiserum, it was found that the ‘inner’ bands arise during the first 4 to 5 days of development (Table 1), that is the period during which the neural plate is formed, neural folding occurs and the primitive brain vesicles differentiate. The antigenic components responsible for the appearance of the ‘outer’ bands appear during the period from the 6th to 12th day, that is during the establishment of the definitive cyto-architecture of the brain. It is thus apparent that the antigenic components which cause the ‘outer’ bands and which seem to be responsible for the effect on embryonic development, are not present in appreciable amounts before the 6th day of development. Hence, it is understandable that brain extracts prepared from embryos younger than 6 days were ineffective when deposited on 24-hr. embryos. Furthermore, it is understandable that brain extracts prepared from the mouse and the rat were ineffective when applied on 24- to 32-hr. chick embryos (Clarke & McCallion, 1959). The brain extracts of these animals appear to have no antigenic components in common with that of the chick when tested against chick brain antiserum. It is evident from Table 3 that the chick brain antigens responsible for the production of the ‘outer’ bands are only present in identical form in brain extracts of birds and in those of some reptiles, but not in those from mammals, amphibians and fish. This indicates that the antigens of the chick brain have a rather high degree of specificity in comparison with those of the chick lens (Langman & Maisel, 1962).

When the various fractions of brain homogenate were separated by means of continuous flow electrophoresis, it was found that the effective component was restricted to those fractions which in the electrical field moved to the positive pole (Lenicque, 1959). Those moving towards the negative pole and containing mainly lipoproteins appeared to be ineffective. This finding is in agreement with our experiments which showed that the brain components collected from the positive pole and tested against brain antiserum formed precipitin bands which
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coalesced with the 'outer' bands. The fractions collected from the negative pole, however, formed precipitin bands fusing with the 'inner' bands. Hence, it is evident that the brain proteins which in the agar plate cause the formation of the 'outer' bands are responsible for the abnormalities of the brain and eye caused by applying adult chick brain extract on 24- to 32-hr. chick embryos. These proteins are the specific antigenic components of the brain, and are found in identical form only in the spinal cord, the peripheral nerves and the nervous retina, but not in any of the other organs or tissues of the chick.

Hence, it seems that the effective brain proteins, though arising only at day 6 of development are already able to interfere with the metabolic process of the cells during the 32- to 96-hr. period, resulting in abnormal growth and differentiation of the nervous system and eye.

SUMMARY

In previous experiments it has been shown that saline soluble extracts of adult chicken brain, when deposited on 24- to 32-hr. chick embryos, cause abnormalities of the brain and the eye. Since neither the soluble components of the brain extract, nor the chemical composition of the embryonic brain cells is known, the present study was undertaken to analyse by means of immunological techniques the soluble fractions of adult chicken brain; to study the appearance of these fractions in the course of embryonic development, and to determine the organ and species specificity of the various brain components. It was hoped that the information thus obtained might give a better understanding on the mode of action of adult tissue homogenates on the corresponding tissue in the embryo. The following results were obtained:

1. Adult brain extract, tested against chick brain antiserum by means of the agar diffusion technique causes the formation of five to eleven precipitin bands, indicating the presence of substantial amounts of five to eleven saline-soluble antigenic brain components. The total precipitin pattern consists of two distinct groups of bands: a group of four to eight rather vaguely defined bands, located close to the antiserum well and referred to as the 'inner' bands, and a group of one to three bands close to the antigen well and referred to as the 'outer' bands (Plate, 1, 2).

2. The antigenic brain components giving rise to the 'inner' bands arise during the first 5 days of development, that is the period during which neural plate formation, neural folding, and differentiation of the primitive brain vesicles occur. They have no organ specific characteristics, since they are present in identical form in all the other organs and tissues of the chick (Tables 1 and 2).

3. The antigens giving rise to the 'outer' bands appear between the 5th and 12th day of development, that is the period during which the definitive cytoarchitecture of the brain is established. They are also present in extracts of nervous retina, spinal cord and peripheral nerves, but are absent in all other tissues
and organs of the chick. Hence, these antigens are highly specific for nervous tissue (Tables 1 and 2).

4. The chick brain antigens causing the ‘inner’ and the ‘outer’ precipitin bands are present in antigenically identical form in brain tissue of the turkey and duck, to a lesser extent in that of some of the reptiles, but are absent in brain tissue of mammals, amphibians and fishes (Table 3).

5. When the soluble components of the chick brain are separated by means of continuous flow electrophoresis and tested against brain antiserum, the most negatively charged fractions are found to form the ‘outer’ bands, while the positively charged molecules form the unspecific ‘inner’ precipitin bands.

RÉSUMÉ

Étude immunologique des effets de l’extrait de cerveau sur le tissu nerveux en cours de développement, chez l’embryon de poulet

Au cours d’expériences antérieures, on a montré que des extraits salins solubles de cerveau de poulet adulte, quand on les dépose sur des embryons de poulet de 24 à 32 heures, provoquent des anomalies du cerveau et de l’œil. Comme on ne connaît ni les constituants solubles de l’extrait de cerveau, ni la composition chimique des cellules cérébrales embryonnaires, on a entrepris la présente étude pour analyser, au moyen de techniques immunologiques, les fractions solubles du cerveau de poulet adulte, pour étudier l’apparition de ces fractions au cours du développement embryonnaire, et pour déterminer la spécificité, relative à l’organe et à l’espèce, des divers constituants du cerveau. On espérait que les renseignements ainsi obtenus pourraient apporter une meilleure compréhension du mode d’action des homogénats de tissus adultes sur le tissu correspondant de l’embryon. Les résultats suivants ont été obtenus:

1. De l’extrait de cerveau adulte, testé avec du sérum anti-cerveau de poulet au moyen de la technique de diffusion dans l’agar, provoque la formation de 5 à 11 bandes de précipitation, indiquant la présence de quantités substantielles de 5 à 11 constituants antigéniques cérébraux solubles dans les solutions salines. Le spectre total de précipitation consiste en deux groupes de bandes distincts: un groupe de 4 à 8 bandes vaguement définies, localisées près de la source d’antisérum et qualifiées de bandes ‘internes’, et un groupe de 1 à 3 bandes situées contre la source d’antigène, et qualifiées de bandes ‘externes’ (Plate 1, 2).

2. Les constituants cérébraux antigéniques donnant naissance aux bandes ‘internes’ apparaissent pendant les cinq premiers jours du développement, c’est-à-dire la période pendant laquelle surviennent la formation de la plaque neurale, le plissement neural et la différenciation des vésicules cérébrales primitives. Ils n’ont pas de caractères organospecifiques, car on les trouve sous une forme identique dans tous les autres organes et tissus du poulet (Premier et second tableaux).
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3. Les antigènes, qui donnent naissance aux bandes 'externes', apparaissent entre le cinquième et le douzième jour de développement, c'est à dire, la période pendant laquelle la cyto-architecture définitive du cerveau est établie. Ils sont aussi présents dans les extraits de rétine nerveuse, d'épine dorsale et de nerfs périphériques, mais ils sont absents de tous les autres tissus et organes du poulet. Donc, ces antigènes sont très spécifiques vis à vis du tissu nerveux.

4. Les antigènes de cerveau de poulet qui provoquent les bandes de précipitine, 'externe' et 'internes', sont présentes sous forme antigénique identique dans les tissus cérébraux de dinodon et de canard, et a moindre degré dans ceux de certains reptiles, mais ils sont absents des tissus nerveux de mammifère, d'amphibien et de poisson (Troisième Tableau).

5. Lorsque les constituants soluble de cerveau de poulet sont séparés par l'électrophorèse à écoulement continu, et testé avec du sérum anti-cerveau, on découvre que les fractions portant les charges les plus négatives forment les bandes de précipitine non spécifiques 'internes'.

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REFERENCE LIST


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