Experimental Neoplastic Formation in Embryonic Chick Brains

by Bengt Källén

From the Tornblad Institute of Comparative Embryology, Lund

WITH TWO PLATES

In mammalian teratology, a malformation consisting of 'overgrowth' of the neural tube has been described by, among others, Patten (1952, 1957) and Ariëns Kappers (1956, 1957). Sjodin (1957) thought it to be a post-mortem effect and not a true malformation. The present author (1955) demonstrated that a similar malformation could be produced in the rostral part of the brain of chick embryos by operations at somite stages on the rostral end of the rhombencephalon which damaged underlying notochordal structures. Bergquist (1959a, b) has discussed this problem further. The overgrowth is made up of an excessive proliferation in the neural epithelium with a reduced cell differentiation, giving rise to only a thin layer of migrated cells but to a marked development of neural epithelium in 4- to 5-day embryos. The vesicles of the hemispheres and mesencephalon are strongly folded, and Bergquist (1959c) has produced evidence that the folding is due to the accumulation of mitoses along the ventricular lining of the vesicles. For further details of this malformation the reader is referred to Bergquist's papers.

In the present paper data are presented which provide evidence for a neoplastic development in the overgrown brain parts.

MATERIAL AND METHODS

Chick embryos at stages between 8 and 18 somites were vitally stained with neutral red, and, after removal of the vitelline membrane, the rhombomere nearest to the rostral part of the brain was extirpated with the aid of glass needles. The eggs were then sealed and incubated. The embryos were killed in Bouin's fluid at ages varying between 3 and 12 days of incubation. All 16 embryos fixed were living at the time of fixation. Serial sections at 10 or 15 μ were prepared and stained with haematoxylin and eosin.

Author's address: Tornblad Institute of Comparative Embryology, Lund, Sweden.

RESULTS

Overgrowth was obtained in nearly all the embryos operated on in this way. The mortality rate was, however, about 60 per cent.

As was stressed by Bergquist, very different degrees of overgrowth may occur in different individuals similarly operated. In rare cases no disturbances could be found macroscopically, and only after examination of the sections could abnormalities indicating overgrowth be seen. In one embryo, for instance, the only abnormality is a folding of the mesencephalon on one side. Usually, however, the disturbance of growth could easily be seen externally and was pronounced in sections. In some cases very localized though well-developed abnormalities were present. In Plate 1, fig. A a hemisphere with such a localized malformation is shown, and sometimes similar conditions could also be found in the mesencephalon.

Bergquist (1959a) has given a detailed description of the changes in morphology in embryos aged 4 to 5 days, and his observations were verified in the present material. A fairly regular neural epithelium may be observed at this stage, abnormal only in its strong folding and high mitotic activity. The present author has, however, also observed numerous mitoses among the lateral cells in the neural epithelium (Plate 1, fig. B), a feature rarely found in normal embryos. Rosette formations in the epithelium as described by Bergquist (1959a) and found in cases of spontaneous overgrowth (Ariëns Kappers, 1956, 1957) were also seen.

In older stages, very different pictures could be seen. In some cases a fairly normal histogenetic development of the brain-wall seems to have taken place, to judge from the haematoxylin-eosin stained slides. An example has already been mentioned and another is given in Plate 1, fig. C, taken from an embryo with a pronounced abnormality of the mesencephalon. The histological picture of the highly malformed brain part is relatively normal. Fibres developing from such tissue are often seen to grow atypically, sometimes forming aberrant nerves which leave the brain surface at abnormal sites.

In many cases, however, the later growth of the 'overgrown' tissue is apparently unorganized. In Plate 2, fig. A the mesencephalon of one embryo is shown, and here the normal morphology is completely lost and replaced by a fairly compact structure. A similar appearance was found in other embryos. The histology of this tissue is very variable. In some parts (Plate 1, fig. D) a fairly normal histology is found with regular strands of cells and fascicles. In other parts, the tumour formation consists of a dense mass of small and uniform cells with few or no signs of differentiation (Plate 2, fig. B). The cells had a tendency to become arranged in rosette formations in many places.

A tendency of the cells to break through the external brain surface and infiltrate into the surrounding mesenchyme could sometimes be seen in embryos with a highly malformed brain (Plate 2, fig. C).
DISCUSSION

The disturbance which causes the 'overgrowth' phenomenon in the present experiments is undoubtedly due to an increased mitotic activity of the neural epithelium, giving rise to the folding and to the poor differentiation of the brain-wall found in young stages. The degree of disturbance and the localization of the overgrowth phenomenon seems to vary considerably in different embryos for reasons which are not yet known. Similarly, the further development of the 'overgrowth' tissue may occur in different ways in different embryos. Sometimes a fairly normal histogenesis of the cells takes place, giving rise to a brain-wall which may be morphologically abnormal but which contains differentiated cell elements. In most cases, in parts of the 'overgrowth', neoplastic-like formations develop with unorganized growth of poorly differentiated cells, which are small, densely packed, and continue mitotic division at a rate which is unknown in normal development for that age.

Cases of invasive growth of the tumour cells have been observed, but it is not clear whether this is an expression of malignancy. During normal development the neural crest cells show the same ability to invade surrounding mesenchyme, and the infiltration observed in 'overgrowth' cells might well be such a change in cell potency and not a true malignant degeneration.

It is, of course, difficult or impossible to try and draw any exact parallels between the neoplastic formations obtained from 'overgrowth' embryos, and similar phenomena from human pathology. The closest parallel would be tumours belonging to the medulloblastoma group. These tumours are supposed to develop from the embryonic granular layer of the cerebellum, a tissue which from a physiological point of view is comparable to the neural epithelium under discussion in the present paper. Some histological features of the 'overgrowth' neoplasms also agree with those of medulloblastoma, such as the tendency towards rosette formation. In other cases, where the cell picture is less anaplastic, similarities to ependymoma tumours can also be seen. The phenomenon of 'overgrowth' may possibly be etiologically related to these tumours.

SUMMARY

After operations on somite stages of chick embryos in the rostral part of the rhombencephalon, a malformation of the rostral part of the brain develops, consisting of so-called overgrowth. The neural epithelium proliferates actively and gives rise to a strong folding of the brain-wall. During later development of such embryos, the overgrown tissue may differentiate in a fairly normal way or may develop into neoplastic formations, consisting of small, densely packed cells. Mitoses occur in such cells. There is a tendency towards rosette formation. Cases of invasive growth into the surrounding mesenchyme have been found. The neoplastic formations are compared to human medulloblastoma tumours.
B. KÄLLÉN

Plate 1
B. KÄLLEN

Plate 2
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REFERENCES

—— (1959c). Personal communication.

EXPLANATION OF PLATES

PLATE 1

FIG. A. Transverse section of the hemisphere of embryo SA, 7d, 23 hours. Note localized tumour formation on left. Haematoxylin-eosin, 10 μ. x15.

FIG. B. Detail of neural epithelium of the overgrown mesencephalon of embryo S6, 4d, 22 hours. A group of mitoses among lateral neural epithelium cells can be seen. Haematoxylin-eosin, 10 μ. x590.

FIG. C. Transverse section of overgrown part of the mesencephalon of embryo S159, 7d, 23 hours. Note fairly normal histogenesis in the malformed brain. Haematoxylin-eosin, 10 μ. x17.

FIG. D. Transverse section through part of the overgrown mesencephalon of embryo S50, 9d, 6 hours. The histological differentiation in this region is relatively normal. Haematoxylin-eosin, 15 μ. x 60.

PLATE 2

FIG. A. Transverse section through part of the overgrown mesencephalon of embryo SA, 7d, 23 hours, showing tumorous malformation in the tectum opticum. Haematoxylin-eosin, 10 μ. x16.

FIG. B. Transverse section through part of the overgrown mesencephalon of embryo SA, 7d, 23 hours, showing tumour cells. Note uniform cell picture of immature nuclei. Mitoses can be seen. Haematoxylin-eosin, 10 μ. x590.

FIG. C. Transverse section of the mesencephalon of embryo S50, 9d, 6 hours. Cells have grown from the tumour tissue into the surrounding mesenchyme. Haematoxylin-eosin, 15 μ. x 140.

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