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A pilot study of cell production by the ganglionic eminences of the developing mouse brain

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INTRODUCTION

The purpose of this paper is to examine the mechanism of cell production in the ganglionic eminences of the developing mouse forebrain by recording the number, location and plane of cleavage of mitotic figures at different stages of development. Similar recordings have already been made in the parts of the mouse neural tube forming the spinal cord, diencephalon and neocortex (Smart, 1972*a*, *b*, 1973), and the background to the present study can be derived from these papers.

The two eminences in the wall of the telencephalon which mark the site of production of the basal ganglion cells lie medial and lateral to each other in the human brain and are so designated in most terminologies. In the mouse, however, where ballooning of the lateral ventricles is less, these eminences lie ventral and dorsal as is seen in Figure 5. In order to keep the terminology consistent, the earlier-appearing ventral eminence at the telo-diencephalic junction will be referred to as the medial ganglionic eminence, and the later-appearing dorsal eminence as the lateral ganglionic eminence. The eminences are composed of two mitotically active cell populations which can be distinguished by using nuclear morphology as the main criterion. As in other parts of the neural tube, there is an *ependymal layer* of elongated nuclei, several nuclei deep, arranged with their long axes at right angles to the ventricular surface. The cytoplasm of the cells to which these elongated nuclei belong presumably extends to the ventricular surface, where it is intercalated into the terminal bar network. The radial arrangement of these nuclei is attributable to the orientation effect of this apical tether. The ependymal layer forms a proliferative compartment with certain interesting characteristics. Typically the nuclei of the ependymal compartment, when about to divide, migrate to the cell apex where the visible stages of mitotis are located (Sauer, 1935). In the segment of the neural tube which gives rise to the spinal cord, apical migration is the rule and mitotic figures are virtually restricted to the surface of the central canal. In the diencephalon, however, mitotic figures have been found in other locations within the layer of radially orientated nuclei (Smart, 1972*b*), and these are considered to be occurring in non-migratory nuclei of the ependymal layer whose cytoplasmic characteristics have yet to be defined. Such activity also occurs in the ependymal layer of the ganglionic eminences and will be referred to as *non-surface ependymal mitosis*. In areas of the ependymal layer where mitotic activity among apically migrating nuclei is high, and the ventricular surface zone is saturated with mitotic figures, early stages of mitosis are also found among the nuclei just below the layer of mitotic nuclei lying immediately adjacent to the

ventricular surface; these are referred to as *subsurface ependymal mitoses*, and are considered to belong to nuclei of tethered cells which are entering visible mitosis before a space is available for them at the ventricular surface.

The non-ventricular surface of the ependymal layer in the ganglionic eminences is in contact with a population of irregularly round or oval nuclei which lack uniform orientation. These nuclei when stained with haematoxylin have the same intensity of staining and internal details as the nuclei of the ependymal layer. This important, mitotically active population will be referred to as the *subependymal proliferative compartment*. This is intended to be a descriptive phrase which leaves open the relationship of this early population to the well known subependymal layer or plate described in a similar location in the adult brain by Allen (1912), and subsequently by many other authors, the most recent study being by Paterson, Privat, Ling & Leblond (1973).

MATERIALS AND METHODS

The material consisted of mouse embryos taken at daily intervals from 10 to 19 days post-conception. The whole embryos, or in the older embryos, the whole heads, were fixed in Carnoy's solution, embedded in paraffin wax, and serially sectioned at 6 μm in the coronal plane. Observations were made on sections passing through the interventricular foramen where the two eminences were separated by a prominent sulcus. At this level recordings were made of the number, location and plane of cleavage of mitotic figures at the different ages in two littermates. For the histological descriptions the whole series of coronal sections, including additional sets of serial horizontal sections, were used to establish the nuclear details, i.e. to ensure that cell nuclei described as 'round' were not oval nuclei cut in a plane at right angles to their long axes. Serial sections of postnatal brains, at intervals from birth to 21 days, similarly fixed and sectioned, were also available for study.

The sets of prenatal sections were the same as those used in previous studies of the histogenesis of other areas of the mouse central nervous system (Smart, 1972*a*, *b*, 1973). The same microscope, lens system and ocular micrometer were also used, a division of the latter being calibrated at 43 μm. The counting procedures carried out were also similar to those used previously and are briefly summarized below. As the results in the littermates were similar, the findings in only one specimen are described. Ages in days, unless otherwise stated, refer to days post-conception.

Surface index

The surface index, i.e. the number of mitotic figures occurring per unit length of the surface of the lateral ventricle, was determined by lining up the scale of the ocular micrometer parallel to the ventricular surface of the developing telencephalon, and recording the number of mitotic figures lying opposite each division of the micrometer scale. The scale was moved progressively along the ventricular wall, from the junction between the diencephalon and the telencephalon to the most superior part of the ventricle. On the curvature of the ganglionic eminences it was found possible to position the scale of the micrometer so that slight rotation brought successive divisions to lie alongside the changing curvature. Even so, some inaccuracy was introduced by measuring the surface of the curvature with a straight line scale. The

inaccuracy was slight, however, as the length of a micrometer division (43 μm) was small in relation to the radius of curvature of the eminences.

The procedure was carried out in five alternate serial sections at each age period. The results of corresponding divisions in each of the five sections were summed and averaged. As about one hundred divisions were involved in each section, the results were condensed as in the previous studies by summing adjacent groups of five divisions and expressing the final result as the average per five-division group (i.e. the average number of mitotic figures per 5×43 μm of ganglionic surface). A five-division group was not allowed to overlap from one ganglionic eminence to another. The average for terminal groups which did not comprise a complete five-division unit was estimated.

No attempt was made to calculate an area index (the number of miotic figures per unit area of the ependymal layer) as in previous studies, as the non-ventricular boundary of the ependymal layer in the ganglionic eminences was not sufficiently well defined.

Location of non-surface mitotic figures

The five sections at each age group which were used for the surface index counts were searched under a × 40 objective for mitotic figures located away from the ventricular surface. The positions of such figures were determined and plotted on an outline diagram of the section.

Orientation of cleavage planes

The orientation of mitotic figures at the ventricular surface was determined by counting the orientation of a minimum of one hundred metaphases, anaphases, and early telophases in each age group. The orientations were assessed according to the angle made by the plane of cleavage with the surface of the ventricle, using the criteria established in a previous study (Smart, 1970). An attempt was also made to classify the orientation of the plane of cleavage in the non-surface mitotic figures occurring within the ganglionic eminences by referring the plane of division to the nearest part of the ventricular surface. In practice, these counts were restricted to mitotic figures occurring within the ependymal layer or in the immediate neighbourhood of its non-ventricular surface.

RESULTS

Ten days post-conception

At this stage there was no evidence of ganglionic eminences. The epithelium of the diencephalon and cerebral vesicle was about 4–6 nuclei deep. In the two specimens studied the surface index (average number of mitotic figures per division of the ocular micrometer) at the curvature of the telo-diencephalic junction was 2·6 and 2·4 respectively. No non-surface mitotic figures were present in this area.

Eleven days post-conception

The medial eminence had appeared as a roughly hemispherical swelling straddling the telo-diencephalic junction. The ependymal layer was about 100 μm deep, and on

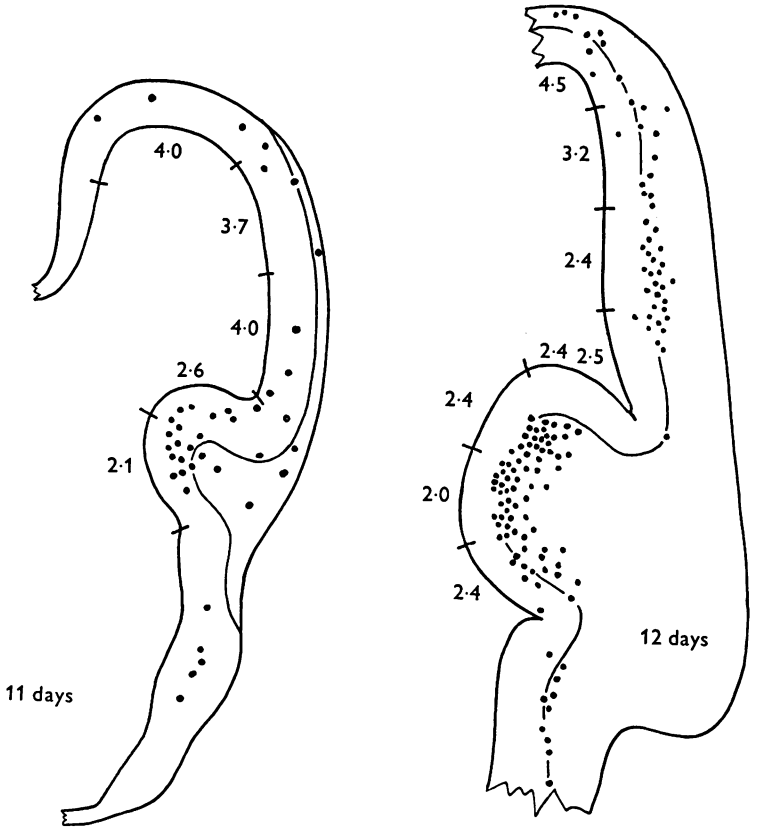

Fig. 1. Outline of coronal section through diencephalon and cerebral vesicle of 11 day post-conception mouse brain at level of interventricular foramen. The ventricular surface is divided into segments, each corresponding to five divisions of the ocular micrometer. The figure opposite each segment is the surface index, i.e. the average number of mitotic figures per division of the ocular micrometer. The black dots indicate the positions of non-surface mitotic figures in five alternate serial sections. The non-ventricular boundary of the ependymal layer is also indicated. Figs. 1–4, 6, 7 and 9 are to the same scale.

Fig. 2. Outline of coronal section of 12 day post-conception mouse brain at level of interventricular foramen. Conventions as in Fig. 1.

its non-ventricular surface it merged with a layer of rounder nuclei, whose staining characteristics with haematoxylin were similar to those of the ependymal layer nuclei. Traced laterally, the nuclei became progressively slightly larger, less granular, more palely staining, and possessed of more obvious nucleoli. These were regarded as belonging to immature neurons. The presumptive site of the lateral eminence in the lateral wall of the cerebral vesicle (Fig. 1) was composed of an ependymal layer of the same thickness as in the medial eminence. This was overlain by a layer of immature neurons continuous with those of the medial eminence.

The surface index over the medial eminence was about 2·5. The specimen in Fig. 1 gave an average of 2·1 for the lower quadrant of the curvature and 2·6 for the upper.

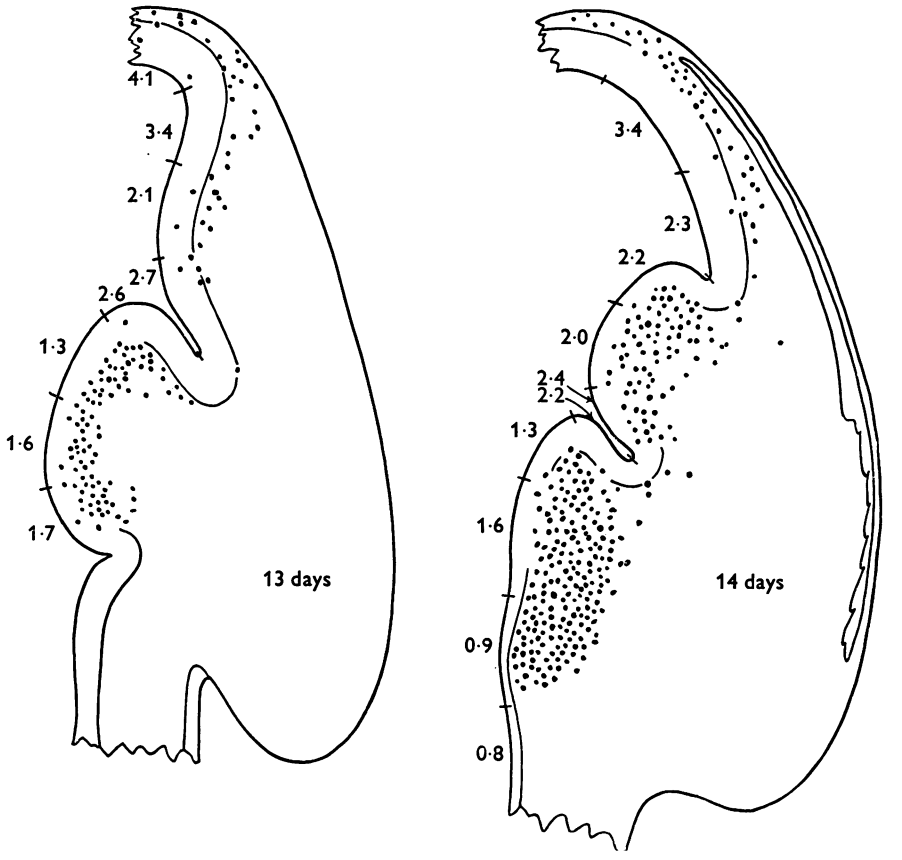

Fig. 3. Outline of coronal section of 13 day post-conception mouse brain at level of interventricular foramen. Conventions as in Fig. 1.

Fig. 4. Outline of coronal section of 14 day post-conception mouse brain at level of interventricular foramen. Conventions as in Fig. 1.

Over the site of the future lateral eminence and over the roof of the cerebral vesicle the value was about 4·0 (Fig. 1).

Non-surface mitotic figures within the medial eminences were scattered principally in the outer part of the ependymal layer (Fig. 1). A few mitotic figures were found among the round nuclei whose staining characteristics were similar to those of the ependymal layer, and these nuclei were regarded as forming the incipient sub-ependymal compartment. A few mitotic figures were also found among the layer of immature neurons. In the sections examined to provide Figure 1 there were 117 surface and 25 (about 17 %) non-surface mitotic figures. At the site of the future lateral eminence, there were some sub-surface ependymal mitoses and relatively few non-surface ependymal mitoses. There was no clear evidence of a sub-ependymal compartment.

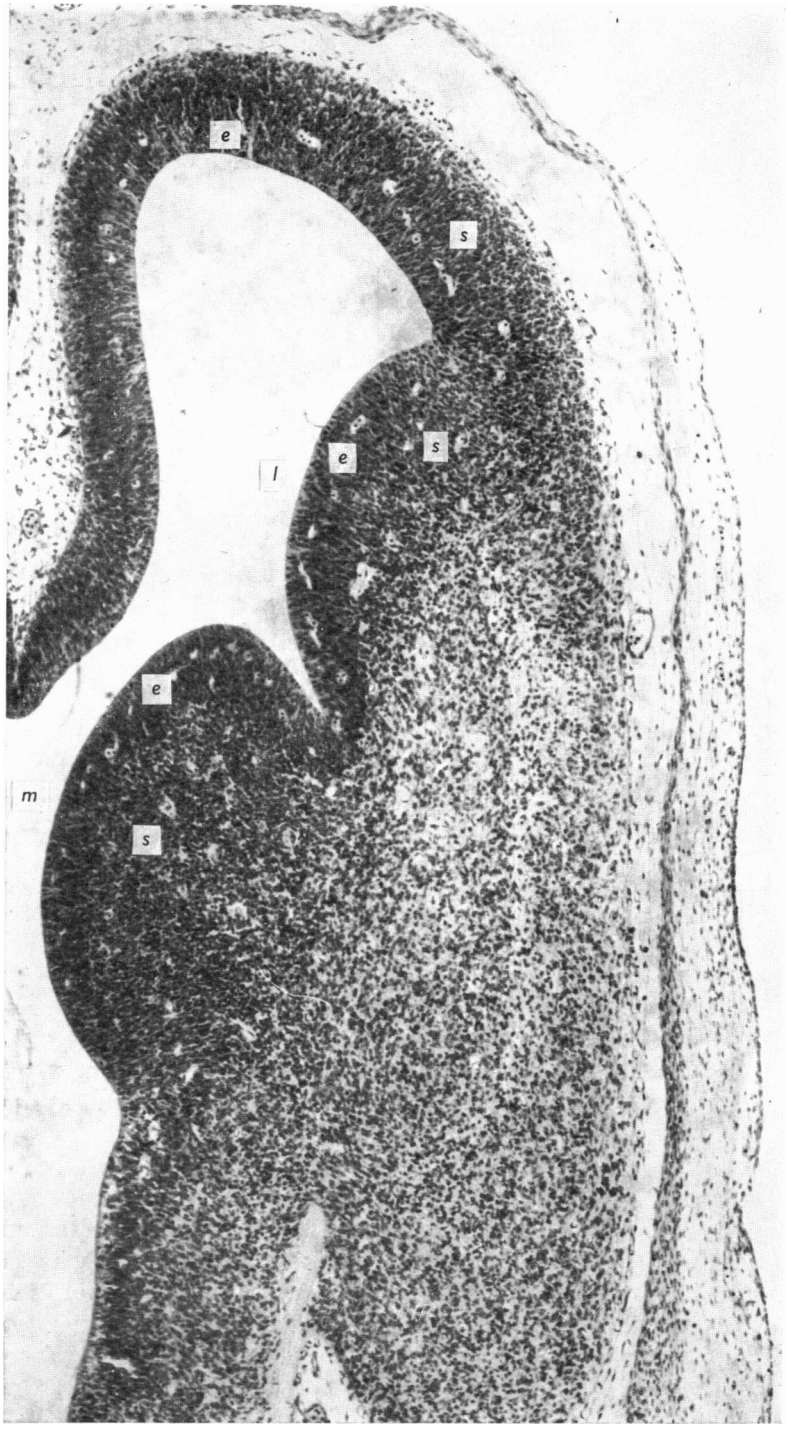

Fig. 5. Photomicrograph of 6 μm haematoxylin and eosin stained section of coronal section through 13 day post-conception mouse brain corresponding to diagram in Fig. 3. *m*, medial ganglionic eminence; *l*, lateral ganglionic eminence; *e*, ependymal layer; *s*, sub-ependymal compartment. $\times 75$.

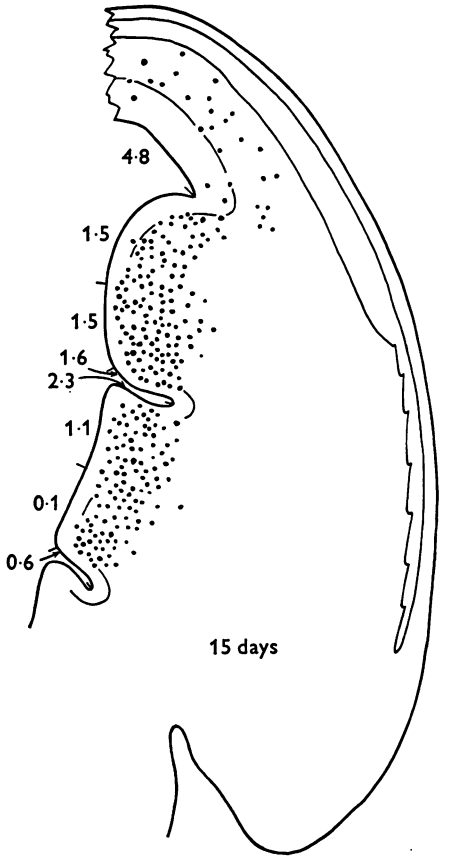

Fig. 6. Outline of coronal section of 15 day post-conception mouse brain at level of interventricular foramen. Conventions as in Fig. 1.

Twelve days post-conception

The medial eminence had become more massive. The thickness of the ependymal layer had not altered appreciably. The space within the increased curvature of the medial eminence was occupied by an increase in the sub-ependymal compartment and in the number of immature neurons, which were now sufficiently numerous to form an obvious bulge in the ventrolateral aspect of the hemisphere (Fig. 2). The lateral eminence was represented by a gentle convexity occupying most of the lateral wall of the hemisphere. It, too, had acquired a narrow sub-ependymal compartment which tapered into the vault of the hemisphere, but was not in continuity with the sub-ependymal compartment of the medial eminence.

The surface index over the medial eminence was about the same as at 11 days (cf. Figs. 1 and 2). Over the lateral eminence it had decreased from about 4·0 to under 3·0. The specimen used for Figure 2 gave values of 2·5, 2·4 and 3·2 running ventro-dorsally. Sub-surface ependymal prophases were infrequent at 12 days and at subsequent ages were virtually absent.

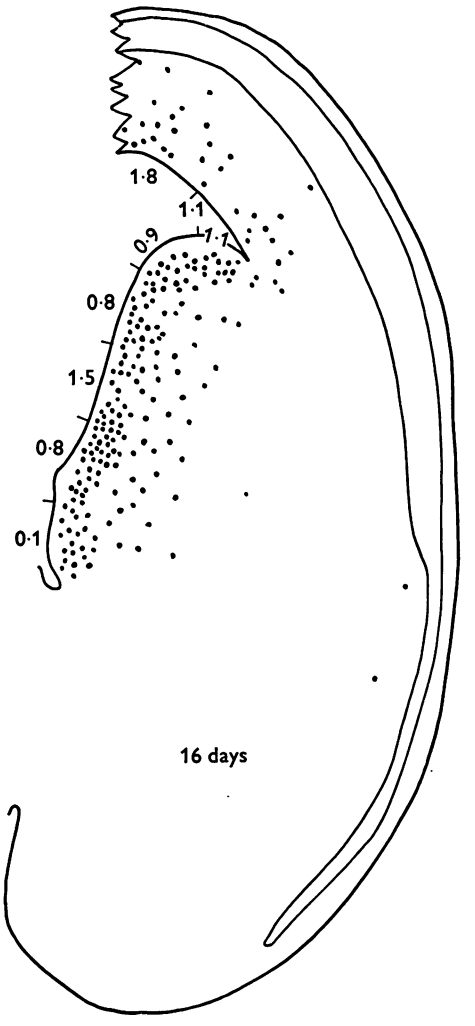

Fig. 7. Outline of transverse section of 16 day post-conception mouse brain at level of interventricular foramen. Conventions as in Fig. 1.

Non-surface figures (i.e. figures which were neither surface nor subsurface) in the medial eminence were now more numerous. In the sections used to construct Figure 2 there were 230 surface figures and 77 (about 25 %) non-surface. The latter were located principally in the (non-surface) outer parts of the ependymal layer and among the nuclei of the sub-ependymal compartment.

In the lateral eminence non-surface figures had appeared in some numbers (Fig. 2). Scattered non-surface figures also extended into the roof of the hemisphere.

Thirteen days post-conception

The internal constitution of the medial eminence was similar to that at 12 days (cf. Figs. 2 and 3). The surface index was, however, lower, being 1·7, 1·6, 1·3 and 2·6,

traced ventro-dorsally in Figure 3. The surface figures in Figure 3 numbered 180 and the non-surface 94, the latter, constituting 34 % of the total.

The lateral eminence was more prominent and associated with a larger sub-ependymal compartment than at 12 days. The sub-ependymal compartment associated with the ventral half of the lateral eminence was less populous than that associated with the dorsal (Fig. 5). In the dorsal half the sub-ependymal compartment extended laterally to abut against a layer of closely packed immature neuron nuclei occupying a position which a day later would be the site of the cortical plate (Fig. 4). Dorsally, the compartment extended past the eminence to be continuous with a thin layer of similar sub-ependymal nuclei extending into the roof of the hemisphere, as at 12 days.

Fourteen days post-conception

At this stage the ependymal layer of the medial eminence had started to decrease in thickness while the sub-ependymal compartment and its associated mitotic figures spread laterally (Fig. 4). In Figure 4 non-surface figures constituted 57 % of the total, outnumbering surface figures by 200 to 150. The lateral eminence continued to maintain an ependymal layer of undiminished thickness with a surface index similar to that at 13 days. The eminence was now demarcated from the hemispheric roof by a sulcus (Fig. 4), although the continuity of the sub-ependymal compartment of the eminence and the vault was maintained. The cortical plate was first evident at this stage.

Fifteen days post-conception

At this age there was a general decline in the thickness of the ependymal layer, and a slight decline in the surface index over both eminences (Fig. 6). The sub-ependymal compartment was undiminished. The mitotic figures within it appeared to be about as dense as at 14 days (cf. Figs. 4 and 6). The non-surface figures in Fig. 6 constituted 53 % of the total.

Sixteen and seventeen days post-conception

There was a further progressive decline in the depth and surface index of the ependymal layer over this period (Fig. 7). The walls of the sulcus between the two eminences had fused and the two sub-ependymal compartments had united. Non-surface mitotic figures, although less densely distributed than at 15 days, were more numerous than those occurring at the related ventricular surface (Figs. 7 and 8).

Eighteen and nineteen days post-conception

The decline in mitotic activity at the ependymal surface continued (Fig. 9). At 18 days a change in the character of the ependymal nuclei was observed; they became slightly larger and paler staining, resembling in appearance the nuclei of the adult ependymal lining of the ventricles. By 18 days the lateral wall of the lateral ventricle had become flattened and the ganglionic 'eminences' disappeared. A diminishing sub-ependymal compartment persisted, and although mitotically less active, it contained an increasing percentage of the total number of mitotic figures owing to the proportionately greater decrease in the mitotic activity of the ependymal layer. The sub-ependymal nuclei retained the staining characteristics of early stages.

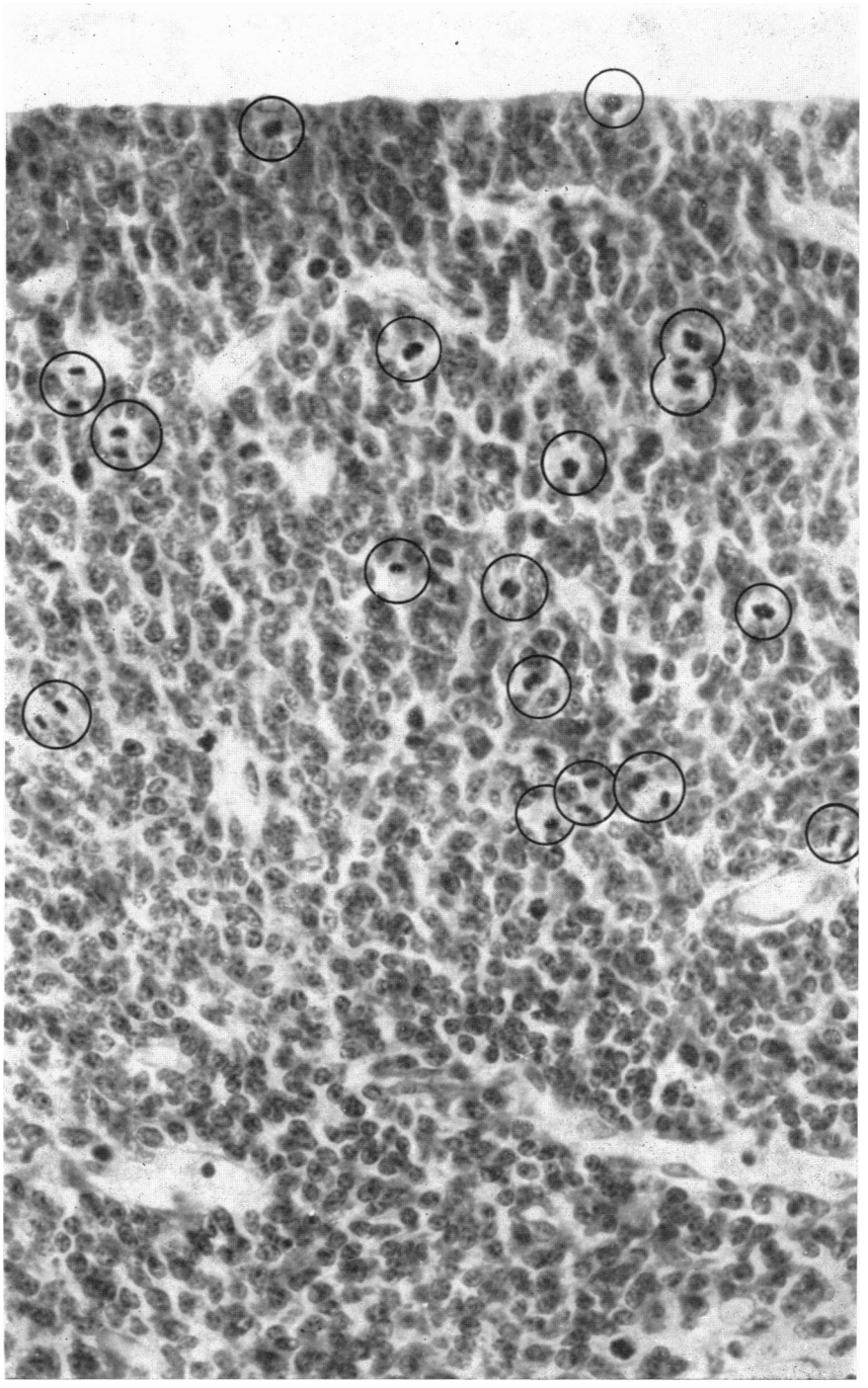

Fig. 8. Photomicrograph of 6 μm haematoxylin and eosin stained section through medial eminence of 16 day post-conception embryo. The ventricular surface is uppermost. Mitotic figures have been circled. Note the scarcity of mitotic figures at the ventricular surface compared with the large number scattered through the sub-ependymal compartment. $\times$ 500.

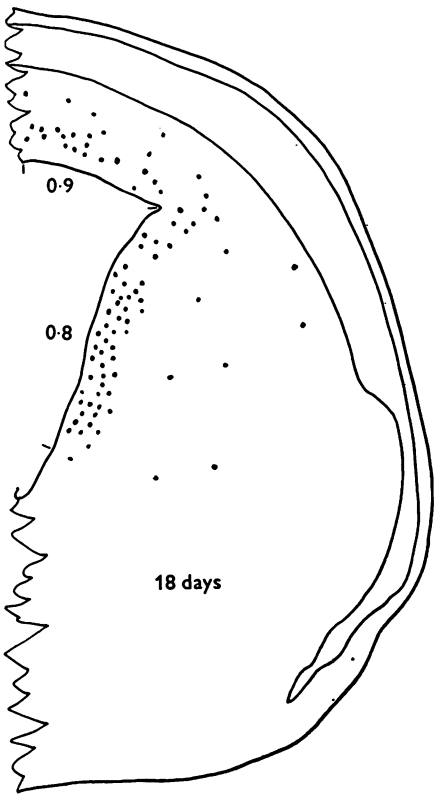

Fig. 9. Outline of coronal section of 18 day post-conception mouse brain at level of interventricular foramen. Conventions as in Fig. 1.

Postnatal history

Although after 18 days post-conception ganglionic eminences were no longer a feature of the lateral ventricular wall, their component nuclear populations persisted. The ependymal layer, which, at birth, was still pseudo-stratified to a depth of two or three nuclei, diminished during the first postnatal week and by the end of the second week consisted of a single layer of cuboidal cells which, in many areas, subsequently became squamous. Fusion of the walls of the lateral ventricle also commenced in the first few days after birth. Mitotic figures, although never completely absent, were not sufficiently numerous to warrant calculating a surface index.

The sub-ependymal compartment persisted, although diminishing each day after birth, until by 10 days postnatum it consisted of a thin layer of polymorphic nuclei related to the roof and lateral wall of the ventricle. It also persisted in those areas where opposing ventricular walls had fused. These cells of the diminished compartment continued to be mitotically active.

Planes of cleavage

Mitotic figures at the ventricular surface at all ages were cleaving in a plane at right angles to the ventricular surface in over 90% of cases. The planes of cleavage of

non-surface figures were difficult to assess because of the curvature of the ganglionic eminences. In counts restricted to the non-ventricular half of the ependymal layer and the immediately adjacent parts of the sub-ependymal compartment, 55–65 % of cleavages occurred parallel to the nearest part of the ventricular surface in specimens up to 14 days. At 14 days, and afterwards, when the sub-ependymal compartment extended further laterally, the planes of cleavage of the mitotic figures relative to the ventricular surface could not be reliably assessed.

DISCUSSION

In the initial paper of this series (Smart, 1972*a*) limiting factors affecting the production of nerve cells by the neural epithelium were examined. One of the major limitations was held to reside in the pseudostratified structure of the neural epithelium. The nuclei of pseudostratified cells migrate to the cell apices on entering mitosis. This produces a situation in which the surface of the central canal (or ventricles) becomes saturated with mitotic figures in areas of high pseudostratification or high mitotic rate. Escape from such ependymal 'choking', without slowing down the rate of cell production, may be achieved by increasing the area of the ependymal layer and/or abandoning apical migration – a situation formalized for heuristic purposes as the 'second rule' (Smart, 1972*a*). The mitotic patterns evolved in the developing diencephalon and occipital neocortex, which can be interpreted as illustrating the operation of the second rule by dispersal of mitotic figures, have been described elsewhere (Smart, 1972*b*, 1973). The mitotic pattern seen in the ganglionic eminences similarly reflects an increase in proliferative capacity. In this situation the site of maximum cell production appears to shift from the ependymal layer to a larger sub-ependymal cell population where the nuclei go into mitosis *in situ* and where the cell bodies are probably not tethered to the ventricular surface by cytoplasmic processes. This development removes a major limitation on cell production, as, once freed from the ependymal layer, precursor cells can proliferate without producing surface congestion.

Having overcome this difficulty the operation of the 'first rule' (Smart, 1972*a*) is rendered less exacting. This rule brought attention to the fact that as mature nerve cells do not themselves divide, their production is delegated to a population of undifferentiated precursors which are mitotically active for only a short period at the beginning of the animal's life span. Loss of cells from this population by differentiation represents loss of cell producing power and, therefore, the longer differentiation is delayed the greater the number of nerve cells which can eventually be produced. To indulge this option leads to problems in 'banking' precursors until they are converted into non-dividing neurons. When the bank is the ependymal compartment, it is governed by the second rule. The evolution of a non-migratory proliferative compartment allows the bank to increase in volume instead of area. More precursor cells can, therefore, be accommodated with the acquisition of this additional dimension. If the sub-ependymal mitotic nuclei in Fig. 4, for example, were restored to the ependymal layer and their cytoplasm re-intercalated into the terminal bar network at the ventricular surface, either the ventricular surface or the degree o

pseudostratification would require to be increased at least two-fold; the former would lead to a floridly convoluted ventricular surface and the latter to ependymal choking.

As elsewhere in the developing neural tube, the proliferative activity of the ependymal compartment can be assessed by reference to the surface index and the number of non-surface mitotic figures present. The surface index, when related to the depth of the ependymal layer, gives an indication of the proportion of nuclei participating in apical migration. In the medial ganglionic eminence it is observed that the surface index reaches a maximum of 2·5, which is low compared with the maximum of 4·0 or thereabouts found in the adjacent neocortical ependymal layer (Fig. 1), or occipital neocortex (Smart, 1973), all of which are of a similar degree of pseudostratification. Sub-surface prophases are also rare, as would be expected in an area with few figures at the ventricular surface. Non-surface figures, however, are numerous at 11 days and by 14 days form more than 50 % of the total. Many of these are frankly located within the outer half of the ependymal layer, while others are ambiguously positioned along its non-ventricular border, and are thus difficult to ascribe with assurance either to the layer itself or to the adjacent sub-ependymal compartment.

The sub-ependymal compartment first appears at 11 days as a small population of nuclei lying within the concavity of the ependymal layer of the medial eminence. In the 12 and 13 day specimens it retains this position while becoming mitotically more populous (Figs. 2 and 3). At 14 days and later this mitotic compartment spreads laterally into the core of the eminences (Figs. 4, 6, 7, 8 and 9). The orientation of the planes of cleavage of the non-ependymal figures also tends to become randomly orientated at this time. This transition seems to represent the freeing of cell production in the subependymal compartment from any direct relationship to the ventricular surface. It is interesting to note that while the sulcus between the two eminences is present it separates the sub-ependymal compartments of the eminences. On the other hand the sub-ependymal compartment of the lateral eminence is continuous with a similar compartment underlying the adjacent part of the roof of the hemisphere (Figs. 3–5). Both underlie areas in which neocortical tissue develops, and it seems possible that some cells may be contributed to the cerebral cortex by the lateral eminence.

Although the sub-ependymal compartment declines towards the end of intrauterine life, it nevertheless still persists in the adult in a vestigial form as the much-described sub-ependymal layer or plate, and its postnatal mitotic activity has been the centre of considerable interest. Postnatally, it seems to produce only neuroglia (Lewis, 1968; Paterson *et al.* 1973). In the early stages of its history the compartment is presumably mainly concerned with neuron production, although even at 12 days post-conception some neuroglial precursors are probably leaving thel ayer, as the small tracts which have developed in the vicinity at this early age have what are almost certainly immature neuroglial nuclei among their fibres.

SUMMARY

Cell production in the medial and lateral ganglionic eminences in pre- and postnatal mouse brains was studied by recording the number, location and plane of cleavage of mitotic figures. The site of maximum cell production shifted progressively

from the ependymal layer to an adjacent sub-ependymal proliferative compartment. In the latter, mitosis occurred without the nuclei participating in the migratory movement to the ventricular surface which is characteristic of the nuclei of the ependymal compartment. The sub-ependymal compartment persisted vestigially into postnatal life as the well known sub-ependymal layer.

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