Proceedings of the Iowa Academy of Science

Volume 13 | Annual Issue Article 37

1906

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A STUDY OF THE CHOROID PLEXUS.

(Abstract)

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With the exception of scant references in the standard anatomies very little material treating of the choroid plexuses is available to the general student, and in fact many questions of interest concerning these structures still remain unanswered. The object of the author has been to review the subject up to date and to give the result of his own investigations. The work was carried on in the Neurological Laboratory of the University of Chicago.

On account of their size and their accessibility the plexuses of the lateral ventricles were used exclusively. Mammalian material was studied from the following forms: white rat, rabbit, guinea pig, cat, dog, sheep, and man.

The number of fixatives adapted to the plexuses is somewhat limited. Bouin's fluid, Carnoy's solution, and acetic sublimate proved most satisfactory. For small animals the best results were obtained by fixing the entire brain. The period of fixation was shortened in all the fluids. The usual methods of embedding in paraffin and sectioning were employed. Many stains were used but the most satisfactory results were obtained with iron haematoxylin followed by acid fuchsin.

The choroid plexuses of the lateral ventricles are due to an ingrowth of the pia mater pushing the mesial wall of the hemisphere into the ventricles. The neural wall is of course preserved but it consists only of a simple epithelium. The plexuses are then thin laminae covered with an epithelium, beneath which is a connective tissue stroma containing an extraordinarily rich network of blood vessels.

In many animals the laminae are smooth but in others they are covered with projecting villi. Between these two extremes are to be found all the intermediate gradations. The guinea pig, mouse, and rat possess plexuses that are smooth. In these forms there are prolongations and projections of the folds, but the typical villi are absent. Villi are scarce in the chicken, duck, and pigeon, more abundant in the hog, while they reach a considerable development in the horse, ox, and especially among crocodiles and some of the selachians. In the rabbit the laminae are very irregularly folded but not villous. Villi are numerous along the free edge of the plexus in the sheep. In man villosities are found but the type is somewhat intermediate.

It is generally believed that the choroid plexuses are largest in the embryonic state and that their volume diminishes as the brain reaches its full development. The model of His for the three month human

foetus shows the plexuses as large swollen glandular organs occupying practically all the space in the lateral ventricles. Along with this anatomical fact has grown the idea that the plexuses furnish some kind of a fluid food necessary to the brain during the embryonic period. Accordingly it has sometimes been called the "cerebral placenta". Whatever the case may be in man it seems that the above description does not apply to all forms. In the white rat the size of the plexuses has been carefully studied at various ages. There is a steady gradual growth from the time of the first invagination until the adult appearance is reached. At no time is there any evidence that the organ is enlarged or distended, or that it fills anything like all the ventricular space.

From a freshly killed animal the plexuses may be removed and examined in cerebro-spinal fluid or in normal salt solution. Not a great amount of detail can be obtained in this way, still it serves as a control for the sections subjected to reagents. The cells appear uniformly granular with nuclei shadowy and cell boundaries indistinct. The most careful examination has failed to show the presence of cilia in the adult forms. They are present in young animals immediately after birth, but it seems reasonably sure that cilia are not present in the adult. At the same time cilia are often noticed on the ependyma which has been torn from the ventricular walls.

Under the influence of fixatives and stains many other details appear. The cross section of a small loop of an adult rat's plexus shows the following structures. In the centre of tth section are one or more capillaries. These consist of a delicate endothelial intima which is strengthened by connective tissue cells and fibrils. Between this adventitia and the epithelium are more connective tissue cells and their processes. The epithelium itself is composed of cubical cells which in cross section average about 10-12 micra in width and 8-10 micra in height. Basal and lateral walls are poorly defined but may usually be distinguished as hazy lines. The free edge of the cell is slightly convex. It consists of a thin apical plate or cuticle. The cytoplasm is finely reticular. In the meshes are deeply staining granules which are often collected into small irregular masses. The reticulation for the most part is more pronounced near the periphery. The nuclei are circular in outline and centrally located.

Embryologically the epithelial cells are derived from the inner layer of the neural tube which also produces the ependymal cells. The ependymal and epithelial cells are therefore parts of the same layer, and this may be plainly seen by tracing the plexus back to where its epithelial covering joins the ependyma lining the ventricle. The two types of cells pass into each other by an easy gradation. The epithelial cells have lost all projections from the base which is so characteristic of the ependymal cells. The epithelial layer has also lost all traces of the neuroglia which normally overlies the ependyma.

The epithelium of the plexus consists of but a single layer of cells. In pathological conditions there may be a proliferation and even a stratified condition, but in normal tissue this seems never to be the case.

Intercellular spaces occur in the ependymal tissues of many animals, but such cavities are wanting between the epithelial cells of the plexus. Gold preparations show that nerves are present in the vicinity of the large blood vessels. These are probably vaso motor fibers.

In regard to capillaries, connective tissues, and endothelium the plexuses from other forms show but little variation from what has been described in the white rat. The greatest differences are found in the epithelial cells. In this layer the rabbit differs strikingly from the rat. The epithelium of the rabbit is characterized by the presence of clear spaces in the cells. They are from 2-6 micra in diameter and very numerous. Their contents are dissolved out by elcohol, ether, and xylol. In ordinary stained sections they show as clear oval or round areas. The contents are of a fatty nature as can be shown by their staining readily with osmic acid and Sudan III. These droplets are largest at the apex of the cell. Sometimes one may be seen lying half within, half without the cell. This shows that they are expelled through the top wall. During this process the nucleus remains normal, but rarely it may be pressed in to one side by the droplet. These droplets have not been found in the plexus of any other form studied. These clear spaces may be seen in the fresh tissue and for this reason it is not believed that they can be due to any error in technique. Since their occurrence is not general in the forms studied it is not believed that they represent the chief secretion of the cells. It is therefore best to consider them as of secondary importance.

A second point in the rabbit's plexus is the development of the parietal structures at the apex of the epithelial cells. In the rat the marginal zone is at best but a double contoured line. In the rabbit however it is wider and composed of filaments placed perpendicular to the surface of the cell and imbedded in some kind of an interstitial substance. This gives the cells the appearance of ciliation. The structure is what Vignon (1) describes as the "bordure de brosse" or filamentous plateau. Cytoplasmic microsomes at the base of the filaments simulate basal bodies. Terminal bars may be seen in cross section at the corner of the cells.

The plexus of the dog differs little from that of the rabbit except by the absence of droplets. The epithelial cells in the guinea pig are characterized by a great many nucleoli.

The preceding description has referred entirely to the adult plexus. If a late foetal or newborn specimen be examined striking differences will be noticed. The epithelial cells differ from those of the adult in three particulars: shape, staining power, and location of the nucleus (2). To illustrate, the cells from the plexus of a one day old rat may be compared with those of an adult. In the one day form the epithelial cells are narrower and deeper. Many measurements give the following averages. Cells from the one day rat are 13 micra in height and 8 micra in

(1). Vignon. Recherches de Cytologie Generale sur les Epithelium. Archiv. de Zool. Ex. et Gen. t IX, p. 371, 1901.

(2). The attention of the author was first directed to this shift in position of the nucleus by Dr. Hatai of the Department of Neurology in the University of Chicago. The entire study began from an investigation of that point.

width. Cells from the adult are 9 micra in height and 11 micra in width. The contents of the cell in the young form are well nigh unstainable at least by ordinary methods. The nucleus stains diffusely and a small amount of cytoplasm at the apex takes up stain but the remainder of the cell remains clear save for a few radiating lines of microsomes. The location of the nucleus in the one day rat is peripheral while in the adult it is central. In the former it averages 7 micra from the base while in the latter it is only at a distance of 2 micra. As the rat grows older a gradual change in all these particulars takes place. The cells widen, begin to take up more stain, and the nucleus moves toward the center. By the seventh day the cells have assumed the adult type.

These same changes in the epithelium after birth have been noted in the cat and rabbit, and doubtless they occur in other forms. The primitive cylindrical condition of the epithelial cells is evidently retained until extra-uterine life is well begun. Whether this means that the cells do not function until adult life cannot be said. It would indicate that their work could not be the same as in the adult.

Thus far we have studied the plexus in what might be termed its resting stage. Careful examination shows that there are always some cells in any plexus that have the appearance of secretory phenomena. For many years there has been a suspicion that the choroid plexuses secreted or at least aided in the secretion of the cerebro-spinal fluid. But until recently the idea remained without much supporting evidence. Capelleti (1) in 1899 made use of a cerebro-spinal fistula to study the effect of drugs on the flow of the cerebro-spinal fluid. In 1902 Pettit and Girard (2) went a step farther by administering drugs to animals and then removing the plexuses to find whether there were any evidences of secretion in the epithelial cells. The writer has made experiments along the same line as these investigators. No claim is made for originality but the results may be of interest since in some cases other forms were used for study.

By cleaning away the musculature from the back of the neck a glass canula may be inserted through the dura into the fourth ventricle and the cerebro spinal fluid secured as it is secreted. Dogs were used for these experiments. In etherized dogs the insertion of the canula is followed by a rapid rush of fluid. This is due to the rapid secretion under the influence of the ether and a consequent accumulation of the fluid. The flow from the ether persists for about thirty minutes but gradually decreases. If 1% pilocarpine now be injected through the femoral vein the secretion becomes stronger and reaches a maximum of 3 to 4 drops a minute. An injection of atropine will now abolish the secretion entirely.

Microscopic examination has been made of the plexuses of dogs and rabbits that had been under the influence of ether for 20 minutes, and also of rabbits, guinea pigs, and rats injected with muscarin. The latter drug was most useful when diluted to 1-500.

(1) Capelleti Archiv. Ital de Biol. XXXVI. p. 299-302, 1901.
(2) Pettit and Girard. Archiv. d' Anat, Mic. tV. p. 213-264. 1902-1903.

In the rat experiments with muscarin did not give decisive results but in the case of rabbits and guinea pigs the results were definite. Often as many as two-thirds of the cells showed evidences of secretion. Normally the epithelial cells of the rabbit are about 6 micra high but after the injection of muscarin the height increases to 12 micra. A differentiation into an outer clear zone and a basal granular zone is rather well marked. Granulations are heavier toward the base of the cell. Clear spaces begin to appear toward the top and rarely does the stainable cytoplasm extend to the upper cell wall. Large masses of granules occur in the upper part of the cell where the lines forming reticulations cross. The nucleus remains globular with a clear outline. The things most striking about these modified cells are their great increase in height and the appearance of so much clear space at the apical end.

Parietal structures are not supposed to take any part in the functional activity of epithelial cells. In case of the extrusion of droplets they may be opened but there is no change in their structure during the process. In the case of the rabbit where there are fatty droplets which we consider a secretion of secondary importance, we can find no evidence of any change in the marginal zone of the cell. In the case of the normal secretion, however, the evidence seems to be that the marginal zone of the cell is modified. The apical wall seems to decrease in thickness as the cell increases in height. We do not believe that it disappears during the secretion but it evidently is considerably modified.

The fact that the flow of the cerebro-spinal fluid is increased by the injection of drugs that produce secretory phenomena in the epithelial cells, justifies the conclusion that the fluid is secreted by the choroid plexuses. No doubt the ependymal cells may have their part in the production but it is certainly a minor one compared to that of the plexuses. Additional evidence that strengthens this conclusion is the occurrence of hypertrophy of the plexuses in certain cases of hydrocephalus. The fact that the fluid differs from serum or lymph is also evidence that is in favor of this view.