Micro- and macrovascular changes as the direct cause of parenchymal destruction in congenital murine hydrocephalus

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Microangiography was used to identify the normal and pathological pattern of cerebral vessels in the hy-3 murine mutant mouse (normal and hydrocephalic) at various developmental stages from birth through 21 days of life. The technique employed allows resolution, in the range of 7 to 10 μ of the surface and intraparenchymal (perforating) microvasculature. Ventricular enlargement causes displacement of primary cerebral arteries, followed by both stretching and a decrease in the caliber of primary, secondary, and tertiary vessels (arterial and venous). Ultimately, there is a reduction in the number and caliber of the microvasculature, resulting in diminished cerebral blood flow and cerebral edema. Tissue destruction leading to ependymal rupture, parenchymal cavitation, and the formation of porencephalic cysts within the edematous parenchyma ensues.

External ventricular drainage, by decompressing the ventricles, resulted in rapid restoration of the filling of the primary and secondary vessels, thereby suggesting the primary role of vascular changes in the production of brain damage. This study offers experimental evidence that early diversion of the cerebrospinal fluid interrupts this chain of events in congenital murine hydrocephalus.

Keywords: microangiography, congenital hydrocephalus, cerebral edema, microvasculature, ventricular decompression, porencephalic cysts

In human congenital hydrocephalus the cerebrospinal fluid (CSF) has been trapped within the ventricular system in a hypertensive state, leading to a sequence of events which, if unchecked, results in irreversible brain damage. Numerous attempts have been made to clarify the pathogenesis of hydrocephalus, but few researchers have tried to correlate the dynamics of changes in vascular morphology with the progression of brain damage. The experimental studies of Dandy and Blackfan have shown that obstruction to the outflow of CSF somewhere along its anatomical pathway is directly responsible for ventricular enlargement. It has also been shown that this ventricular enlargement occurs at the expense of the surrounding tissue, most notably the white matter, which becomes markedly thinned. The most common changes found in the parenchyma of the hydrocephalic brain are atrophy, pallor...
and swelling, vacuolation and chromatolysis of nerve cells, hypertrophy of astrocytes, axonal demyelination and degeneration, and a decrease in synapses. It has been suggested that these changes are the result of an increase in intracranial pressure which induces a diminution in cerebral blood flow (CBF) in the hydrocephalic brain. Diminished CBF has also been shown to be present in “normal-pressure” hydrocephalus.

Early observations of the brain vasculature in human hydrocephalus by Penfield and Elvidge in 1932 were contradictory. They stated that, “there may be a decrease in the intramedullary capillary bed. This decrease is only a supposition, not a proven fact, but it seems likely that vascular obliteration begins in the smallest branches of the vascular tree and that further passage of blood through these branches is prevented by their compression without thrombosis and congestion as in other forms of vascular occlusion.” Hassler and De studying experimental hydrocephalus induced by injecting adhesive agents into the subarachnoid spaces or the ventricular system, found a significant loss of smaller vessels (capillary and precapillary) around dilated ventricles, and concluded that ischemia is responsible for the associated changes in all structures along the ventricular surface. The variability of observations in experimentally-induced hydrocephalus is well known, and probably results from lack of uniformity of species (mongrel dogs and cats), of technique (kaolin, lamp black, surgical obstruction), and of reproducibility. The most serious limitation is that these are experimentally induced, not naturally occurring, changes in CSF dynamics and brain damage.

In our laboratories we have a strain of mice (hy-3/hy-3) with congenital hydrocephalus; this provided us with the opportunity to study, in a controlled manner, the progression of the hydrocephalic process as it occurs “naturally” in the mammalian brain, independent of artifact and variations that occur when it is produced experimentally. Our previous publications on the study of these hydrocephalic mice have reported the genetic characteristics, light and electron microscope changes, alterations in CSF production and circulation, and changes in extracellular space.

The sequence of events resulting in end-stage cerebral damage from progressive ventricular dilation has not been documented with regard to changes of the macro- and microvasculature or the effects of these changes upon parenchymal edema, cavitation, and destruction.

The present study was undertaken to identify the role of secondary vascular changes in the pathogenesis of congenital murine hydrocephalus. Available methods for studying the vasoarchitectonics of the brain surface and underlying tissue were used. Microradiography was employed as early as 1913 by Pierre Coby, but the first attempts to use it in the demonstration of capillaries were made in 1935 by Grechiskin. This technique has been perfected during the last few decades. It is suitable for the preparation of thick sections to demonstrate vascular pattern. The most useful x-ray contrast medium is Micropaque, which consists of particles of barium sulfate measuring less than 0.5 μ in diameter.

Materials and Methods

Twenty hy-3/hy-3 mice with congenital hydrocephalus, 5 to 21 days old, were studied. Normal litter mates were used as controls to describe the normal distribution of cerebral arteries in the mouse brain. Thus changes in the vascularization of the hydrocephalic brain could be correlated with gross anatomical, light microscopic, and electron microscopic findings.

The hydrocephalic mice were anesthetized by an intraperitoneal injection of 0.1 ml of 3% chloral hydrate and then injected subcutaneously with 1000 units of sodium heparin. After the onset of suitable sedation, the chest was opened rapidly under the dissecting microscope and a 30-gauge needle inserted into the left cardiac ventricle. The animal was perfused for 10 minutes with Ringer’s solution, which contains 25 units of heparin per 50 ml, and then for 10 minutes with 7.5% Micropaque in distilled water. A bottom-delivering flask (Fig. 1) containing a 7.5% solution of Micropaque maintained in suspension by a magnetic stirrer was connected to intravenous tubing which passed...
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through an ultrasonic mixer and a suspension trap before being attached to the cardiac cannula. The superficial cerebral vasculature was examined grossly under a dissecting microscope to estimate the adequacy of filling with x-ray contrast medium. When perfusion was completed, the mouse was decapitated and the skin removed from the skull. The head was preserved by freezing in acetone, which was at the temperature of dry ice (-79°C). Coronal and sagittal sections of approximately 1 to 2 mm were cut with a razor blade. These sections were immersed in acetone at dry ice temperature for 2 days. When this tissue was removed from acetone, it was placed in a desiccator in propylene oxide, and then embedded in an epon-araldite mixture. The contact micrographs were recorded on Dupont film, using a Sorinson x-ray machine equipped with a Machlett A-2 diffraction tube. Thus we were able to obtain a true tomographic view, in sagittal and coronal planes, of the cerebral vasculature (Fig. 2).

Results

Normal Arterial Pattern

In the normal mouse, as in man, two large vessels, namely the internal carotid and the vertebral arteries, supply the brain on each side. Each internal carotid artery reaches the base of the brain by passing along the lateral side of the hypophysis. After giving small branches to the structures at the base of the brain, they bifurcate into two main branches, the anterior and middle cerebral arteries. The posterior communicating artery runs superiorly and caudally to join the posterior cerebral artery, thus closing the circle of Willis. The basilar artery is formed by two symmetrical vertebral arteries which join at the junction between the medulla spinalis and the medulla oblongata. At this junction, the basilar artery gives off the large inferior cerebellar artery which supplies the posterior and lower part of the cerebellar hemispheres (Fig. 3 left).
The terminal branches of the basilar artery are the two posterior cerebral arteries. These vessels pass dorsally around the brain stem to enter the cleft between the cerebral hemispheres and the brain stem. Each posterior cerebral artery gives off a superior cerebellar artery, which supplies the superior aspects of the cerebellar hemispheres. The anterior cerebral artery runs rostrodorsally to the optic nerve and anterior optic chiasm, where the arteries join to form a single midline vessel (Fig. 3 upper right). This single vessel runs between the hemispheres superiorly and passes dorsally and then caudally over the corpus callosum. It gives off branches superiorly and inferiorly, mainly to the medial aspect of the hemispheres, but to some extent over the convexity of the brain.

The main trunk of the middle cerebral artery gives perforating and lenticulostriate branches before it passes over the cerebral hemisphere where it divides into superior and inferior branches (Fig. 3 lower right). These branches form a leptomeningeal arterial network (from which the short cortical vessels originate) and the typical palisading pattern of cerebral cortical vasculature and transcerebral long vessels. The latter penetrate the cortex without branching, supply mainly cerebral white matter, and end in the subependymal white matter bordering the lateral ventricles.

Cerebral Vasculature of the Hydrocephalic Mouse

In the hydrocephalic mouse the vascular changes depend upon the duration and severity of the hydrocephalic process. The observed ventricular and parenchymal changes in the progression of hydrocephalus may be divided into three stages.

Stage 1 (7 to 14 days of life). This stage is characterized by a moderate dilation of the ventricular system (Fig. 4 upper), a patent aqueduct, and the free flow of CSF through the entire ventricular system and into the basal cisterns. CSF does not flow into the subarachnoid spaces over the cerebral hemispheres. The microangiogram (Fig. 4 right) demonstrates that the anterior cerebral artery is under tension and is displaced by the enlarged frontal horns of the lateral ventricles. The caliber of the anterior cerebral artery is diminished and the number of large branches given off is decreased. The typical palisading pattern of the cortical vessels is preserved; however, there is displacement and irregularity in the architecture of the long transcerebral vessels that supply the underlying white matter. Vascularization of the brain stem is preserved, although there is a decrease in the caliber of perforating branches arising from the basilar artery. The quadrigeminal and superior cerebellar cisterns are enlarged.

Stage 2 (14 to 18 days of life). Disproportionate dilation of the occipital horns of the lateral ventricles causes compression of the quadrigeminal plate. Penetration of the ependymal wall and entry into the surrounding white matter by CSF are increased, and result in the formation of porencephalic cyst.

The microangiogram in Fig. 5 left shows a further decrease in caliber of the middle cerebral artery and its displacement laterally against the skull. The middle cerebral artery's perforating branches (the lenticulostriate arteries) supply the basal nuclei and white matter of the frontoparietal region of the hemispheres. They are displaced inferolaterally, stretched, and narrowed. Their caliber is thereby diminished, although the vascularization of the basal nuclei is well
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Fig. 3. The normal mouse brain after perfusion with India ink. Left: Basal view, showing the distribution of the major vessels at the base of the brain. 1 = internal carotid artery; 2 = anterior cerebral artery; 3 = middle cerebral artery; 4 = basilar artery; 5 = vertebral artery; 6 = posterior inferior cerebellar artery. Upper Right: Midsagittal section demonstrating the distribution of the anterior cerebral artery (1) and collicular artery (2). Lower Right: Lateral view of the cerebral hemisphere showing the distribution of the middle cerebral artery (arrows).

preserved. (Compare this with Fig. 5 right, which illustrates the normal condition.) There is an avascular area in the subcortical white matter of the parietal region, lateral to the ventricles, supplied by long lenticulostriate branches. It is in this region that CSF begins to collect. It passes from the ventricular system and forms a porencephalic cyst separated from the lateral ventricle by a septum of ependyma and subependymal vessels. The vessels around the ventricles and the porencephalic cyst are stretched and run parallel to the wall. Between the hemispheres, at the vertex, there is evidence of a collection of fluid, arteriographically expressed by the presence of an avascular area with lateral displacement and tension on the terminal branches of the anterior cerebral artery. Cortical vascularization of the frontoparietal region is preserved.

The microangiotomogram in Fig. 6 demonstrates more severe dilation of the lateral ventricles. There is a decrease in the vascularization of the cerebral cortex with distortion of the typical palisade pattern of the cortical vessels and their displacement upward toward the skull. The posterior cerebral artery is displaced posteriorly and its branches, which supply the posterior part of the occipital lobe, are stretched and diminished in number. In spite of these changes, the vascularization of the brain stem and the cerebellum is well preserved.

The most involved area of the brain at the end of the second stage of hydrocephalus is the occipital region. The occipital pole of the lateral ventricle is disproportionately dilated and the cerebral mantle is diminished by approximately 75%. The enlarged ventricles press downward on the quadrigeminal plate and the brain stem. This is indicated by changes in the course of the posterior cerebral artery, which becomes triangular in form as it loses its normal rounded appearance (Fig. 7 left). This should be compared with the normal artery shown in Fig. 7 right.

The electron micrograph in Fig. 8, taken from an animal in the second stage of
**FIG. 4.** Upper: Gross sagittal section of the hydrocephalic mouse brain in the first stage of the hydrocephalic process. Note moderate dilation of the lateral ventricles (LV). Right: Sagittal microangiogram taken from the beginning of the second stage of the hydrocephalic animal. Note the attenuation and stretching of the anterior cerebral artery (1) over the lateral ventricle (LV) and the posterior cerebral artery (2), the displacement downward of the branches of the quadrigeminal arteries (3), and fluid collection in the quadrigeminal cistern (FC). × 7.

**FIG. 5.** Coronal microangiograms. × 5. Left: An animal in the middle of the second stage of hydrocephalus, taken at the level of the distribution of the middle cerebral artery (1). It shows the early development of the porencephalic cyst (2). Note preservation of the palisading pattern of the cortical vessels (3). The lenticulostriate arteries (4) are pushed inferiorly and laterally and all of the cerebral vessels are decreased in caliber. LV = lateral ventricles. Right: The normal distribution of the middle cerebral (1), lenticulostriate (2) and cortical vessels (3).

**FIG. 6.** Sagittal microangiogram from a 16-day-old animal demonstrates more advanced enlargement of the lateral ventricles (LV). There is stretching of the anterior cerebral artery (1) and a decrease in caliber, plus a parallel course, of the vessels passing along the walls of the ventricles (2). Note the distortion of the typical "palisade" pattern of the cortical vessels (4), the decrease in number and in the size of the vessels of the occipital area (3), and the displacement downward of the cerebellar vessels (5). × 6.
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hydrocephalus, demonstrates an increase in extracellular space (ECS) to between 40% and 50% in the hydrocephalic brain from approximately 15% in the normal brain. This increase is the result of the flow of CSF which penetrates the ependymal wall as it streams toward the ECS of the cortex. Electron micrographs of the subependymal capillaries show that the enlarged extracellular spaces around the vessels contain particles of the lanthanum tracer which had been injected into the lateral ventricle prior to perfusion. We have noted no evidence of changes in the structure of these capillaries.

Stage 3 (beyond 18 days of life). The edema previously restricted to the white matter now extends into the gray matter of the occipital lobe. A spontaneous ventriculostomy develops within the occipital lobe, connecting the ventricular system with the patent subarachnoid spaces along the vertex of the cerebral hemispheres.

The microangiogram in Fig. 9 shows caudal displacement of the branches of the posterior cerebral artery. In the occipital lobe there is a large porencephalic cyst. The vessels around the lateral ventricle and porencephalic cyst are stretched as they pass parallel to the surface of the cavities. In the cerebral cortex the primary and secondary vessels are markedly displaced. There is a significant loss of the cortical capillary network. The number of tertiary vessels is reduced. The palisade formation in the cerebral cortex is completely disrupted. Brain stem vessels seem to be preserved quite well, in spite of displacement of these structures toward the foramen magnum.

External Ventricular Drainage in the Hydrocephalic Mouse

In the second stage of hydrocephalus external ventricular drainage (EVD) was performed. The occipital horn of the lateral ventricle was tapped. There was spontaneous outflow of CSF under pressure. After this procedure the surface of the brain was observed through the almost transparent skull under a dissecting microscope for 20 minutes. Simultaneously with the onset of brain pulsations, the number of visible cortical arteries increased and the cortical veins and sagittal sinus decreased in size. We have no explanation for the diminution in the size of the sagittal sinus. After this observation period, the animals were perfused with microbarium.

The vasculature before drainage is narrower, stretched, and rectilinear (Fig. 10 left), while in another hydrocephalic animal from the identical stage of hydrocephalus after drainage the appearance of the vessels becomes normal (Fig. 10 right). The remarkable observation is that the diminution in intraventricular pressure (resulting from the external ventricular drainage) is associated with a return to normal of the caliber, form, and course of the cerebral vasculature.

Discussion

Microangiography is a valuable technique for the study of pathological changes in the architecture of the brain. It offers an opportunity to study not only the changes in the primary and secondary vessels of the brain, but also the tertiary vessels and the microcirculation. Thus it was possible for us to study accurately the relationships between brain structures, the vascular system, and the alterations in these relationships caused by hydrocephalus.

From these observations of the changes in cerebral angioarchitecture, and those observed in previous studies on murine hydrocephalus, we can postulate a sequence of events leading to irreversible brain damage. Hydrocephalus in the hy-3 mouse is already apparent in the first few days of life. Initially, there is an absence of the subarachnoid space over the majority of the anterior cerebral convexity, associated with communicating hydrocephalus. Within 14 days of life, an enlarging ventricular system is associated with progressive cerebral edema, involving the gray and white matter of the hemispheres and brain stem. In most animals communicating hydrocephalus is followed by aqueductal occlusion. Whether this aqueductal occlusion is a result of purely mechanical forces, or of a more complex series of both cellular and extracellular pathophysiological processes which result in irreversible morphological changes of the ependymal (and the periaqueductal gray) fine structure and sealing of the aqueduct, is not yet clear. We favor the latter possibility.

An increase in intracranial pressure is associated with these disturbances of CSF circulation, factors which may be responsible for
the progressive dilation of the ventricular system and pathological changes in the surrounding neural tissue.7,8,12,24,34,49

Transependymal CSF perfusion is a compensatory mechanism, a response to the high intraventricular pressure.61,62 It may well occur at much slower rates and lower volume in the normal state. Electron-dense tracers reach parenchymal capillaries by way of the extracellular space (ECS) of the cerebral cortex within minutes after intraventricular injection. This fluid is under a pressure gradient which is transmitted into the ECS and against the cerebral vasculature. Thus, CSF and ECS, under a high head of pressure, compress cerebral vessels as they displace and deform them, causing their caliber to diminish, and presumably resulting in changes in CBF.

Conclusions reached by others16,18 regarding the dilation of the central canal of the spinal cord in experimentally-produced hydrocephalus are not necessarily to be excluded, although we must remember that in this paper we are discussing hereditary hydrocephalus. In point of fact, we, too, have observed that the central canal dilates, just as Gardner stated in his many publications on this subject.14
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The first vascular change in the development of congenital murine hydrocephalus is a decrease in the caliber of the cerebral vessels. This occurs in both white and gray matter, and is progressive. Ultimately, there is a decrease in the number of the secondary and tertiary vessels, which begins in the white matter but rapidly involves the vasculature of the cerebral cortex. Following a decrease in the caliber of vessels there is a disappearance of the normal, “palisade” pattern. The edema and atrophy of periventricular white matter result from the rising pressure of the intraventricular CSF streaming across the ventricular wall and probably to ischemia following compression of the capillaries.

The following sequence of events leads to irreversible brain damage in the hydrocephalic brain: 1) accumulation of intraventricular CSF under pressure; 2) increased transependymal CSF perfusion; 3) parenchymal vascular compression; 4) ischemia; 5) edema; and 6) tissue destruction. The onset of irreversible brain damage in our animals became obvious with the formation of porencephalic cysts within the white matter during the end of the second stage of hydrocephalus. From these observations we conclude that decompression of the intraventricular pressure by ventricular shunting after the second stage of hydrocephalus offers no hope of recovery of cerebral function.

At the beginning of the second stage of hydrocephalus, when early edema of the white matter and enlargement of ECS are present, and there is only a decrease in the caliber of cerebral vessels with displacement of the primary and secondary vessels, drainage of CSF has a beneficent effect. External ventricular drainage at this stage results in a noticeable improvement in cerebral vascularization. A significant increase in caliber of the cerebral arteries after ventricular drainage suggests that the increase in intracranial pressure associated with progressive hydrocephalus is transmitted from the ventricles by the ECS to compress, deform, and displace the cerebral vasculature and, therefore, results in diminished cerebral blood flow. These changes are compounded by the progressive cerebral edema.

These observations lend support to the importance of performing ventricular decompression early, when the process is reversible. They may be directly correlated with our clinical observations that the earlier a hydrocephalic child is shunted, providing the shunt remains functional, the greater the chances are for the patient to attain normal intellectual and motor function. Also they are similar to our observations that the prognosis of hydrocephalus, again as measured by using psychomotor parameters, may be predicted by using cerebral angiography to identify whether there is a full complement of secondary and tertiary cerebral vessels (good prognosis), irrespective of the thickness of the cerebral mantle. The presence of porencephaly in human congenital hydrocephalus invariably indicates that the child will suffer severe psychomotor retardation.

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Summary

1. Changes in the hy-3/hy-3 mouse brain vasculature studied with the microangiotomographic technique are similar to those observed by cerebral angiography in human congenital hydrocephalus.

2. Vascular changes are displacement and deformity of primary arteries; stretching and decrease in caliber of primary, secondary, and tertiary vessels; and disappearance of tertiary vessels.

3. These changes in vascularization were observed first in the white matter and later in the gray matter, and developed pari passu with changes in fine structure.

4. The most involved areas of the brain were in the distribution of the posterior cerebral artery, and consisted of disruption of brain parenchyma with formation of a porencephalic cyst and then spontaneous ventriculostomies in the occipital lobe.

5. After external drainage in the second stage of hydrocephalus, general dilation of the brain vasculature was observed, this suggests improved cerebral circulation in the cortical vessels and indicates that at this stage the process may be reversible.

References

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