Histopathological study of major intracranial arteries in premature infants related to intracranial hemorrhage

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The incidence of various types of intracranial hemorrhage (ICH) and the microscopic appearance of the major intracranial arteries were investigated in 112 premature infants, and related to birth weight. In none of the premature newborns, regardless of the presence of ICH, did the intracranial arteries contain true elastic elements. The wall of the basilar artery was significantly thinner in all premature infants with any kind of ICH. Within each body-weight group those with ICH had intracranial arteries with fewer reticular fibers than did those without ICH. The density of the reticular fibers in the cerebral arteries of premature neonates without ICH did not seem to depend on maturity. The authors believe that the hypoplasia of the major intracranial arteries and the reticular fiber deficiency most probably involve the whole vascular system of the brain and may predispose to hemorrhage.

KEY WORDS • cerebral artery • hypoplasia • reticular fiber deficiency • intracranial hemorrhage • premature infant

Intracranial hemorrhage (ICH) is one of the conditions most frequently contributing to death in newborn infants. The occurrence of ICH of newborns seems to be closely related to immaturity and respiratory failure, suggesting that these factors might play an important role in its pathogenesis. In the majority of cases, ICH occurs in premature infants, especially in those with hyaline membrane disease. However, a significant proportion of premature infants with hyaline membrane disease do not develop ICH. This fact suggests the existence of an independent disorder which may predispose to ICH.

Little information is available about the histological structure of the intracranial arteries of premature infants. In this study, we have examined histologically the walls of the major cerebral arteries obtained from 112 premature newborns at autopsy, and have compared our findings in premature infants with and without ICH. We present our results and discuss possible predisposing factors for the hemorrhage.

Clinical Material and Methods

This study reviews material obtained at autopsy from 112 consecutive infants, with a birth weight ranging from 700 to 2500 gm. This series did not include twins, stillborn babies, infants with malformations, or those with Rhesus isoimmunization. Premature infants who survived more than 5 days were also excluded from the study. Since the gestational age was uncertain in some cases, the infants were divided by their birth weight into four groups: 700 to 1000 gm (26 cases), 1001 to 1500 gm (46 cases), 1501 to 2000 gm (25 cases), and 2001 to 2500 (15 cases).

In each case, general autopsy was performed 4 to 5 hours after death. The lungs of every infant were investigated microscopically. After gross examination and fixation in 10% formalin solution for 1 week, the brains were sectioned coronally at 1-cm intervals. Representative blocks were cut through the caudate nucleus and choroid plexus levels. If it was necessary for the establishment of the exact origin of ICH, serial sections were made. The basilar artery was subjected to microscopic investigation in every case, and in 85 cases all the other major intracranial arteries were examined microscopically. Sections were stained with hematoxylin and eosin (H & E), orcein, trichrome, or Gomori's method for reticulin.

Serial cross sections were made from the middle one-third of the basilar artery. Because of the frequency of manipulative damage to the adventitia, only the thickness of the media together with the intima was measured. Measurements were made without previous knowledge of the presence of ICH. Measurements from...
the narrowest portions of the arterial wall were used, since these were least dependent on the degree of constriction of the vessel walls, and using these helped to exclude differences due to tangential sectioning. Measurements were performed on every 10th section, and in this way 15 sections were evaluated in each case.

The incidence of various types of ICH in each of the four birth-weight groups and the association of ICH with respiratory failure were investigated. The microscopic structure of the major intracranial arteries and the thickness of the wall of the basilar artery were compared in each birth-weight group for infants with and without ICH.

Statistical analysis was performed using the Student t-test and chi-square test.

Results

Figure 1 shows the incidence of ICH by birth-weight groups, and also indicates those cases with intraventricular hemorrhage (IVH). According to many authors, the occurrence of ICH, especially associated with IVH, is correlated with immaturity. Simultaneous hemorrhage from different sources could be observed in each birth-weight group. The coexistence of either bilateral subependymal bleedings (SEH's) or unilateral SEH and subarachnoid hemorrhage (SAH) was the most frequent finding. Naturally, isolated SAH could not be verified in cases of IVH. In the present series, no subdural or intracerebellar hemorrhage was found.

The general autopsy disclosed some kind of pulmonary disease in all cases, including hyaline membrane disease, hemorrhage, pneumonia, aspiration, and atelectasis. Only in the second birth-weight group (1001 to 1500 gm) was the incidence of hyaline membrane disease significantly higher in babies with ICH than in those without ICH (p < 0.05). There was no significant correlation between the incidence of ICH and that of pulmonary hemorrhage in any group. To our surprise, in the fourth birth-weight group (2001 to 2500 gm) the mean birth weight of infants with ICH was significantly higher (p < 0.05) than that of infants without ICH. Except for this, the mean birth weight and the mean gestational age of infants with ICH did not differ significantly from those infants without ICH (Table 1). Despite these data, in each birth-weight group the wall of basilar artery (not including the adventitia) was significantly narrower in infants with ICH than in those without hemorrhage (Table 2).

Light microscopy studies were unable to reveal the number of reticular fibers in the subependymal blood vessels in these specimens. The major intracranial arteries showed normal structure when sections were stained with H & E and orcein. Except for the branching points, the internal elastic lamina appeared as a homogeneous band. The internal elastic lamina always stained blue with trichrome. With these usual staining methods there was no difference between the arteries of infants with and without ICH.

The sections stained by Gomori's method for reticulin demonstrated three distinct patterns of reticular fibers in the media of premature infants. In most infants with ICH, especially associated with IVH, the occurrence of ICH, especially associated with IVH, is correlated with immaturity. Simultaneous hemorrhage from different sources could be observed in each birth-weight group. The coexistence of either bilateral subependymal bleedings (SEH's) or unilateral SEH and subarachnoid hemorrhage (SAH) was the most frequent finding. Naturally, isolated SAH could not be verified in cases of IVH. In the present series, no subdural or intracerebellar hemorrhage was found.

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The sections stained by Gomori's method for reticulin demonstrated three distinct patterns of reticular fibers in the media of premature infants. In most infants without ICH, the media of all major intracranial arteries was supplied with a dense network of fine reticular fibers in a fairly uniform distribution (Figs. 2 left, 3 left, and 4 left). The second pattern also showed a uniform distribution but there were considerably fewer reticular fibers (Figs. 2 right and 3 upper right). The third pattern was characterized by an irregular distribution of reticular fibers, being conspicuously sparse in the outer part.
of the media (Figs. 3 lower right and 4 right). The latter two forms showing partial lack of reticular fibers were seen predominantly in the arterial wall of infants with ICH (Fig. 5). The partial lack of reticular fibers confined to the outer part of the media was the most frequent pattern, occurring in two-thirds of patients. The various forms of deficiency in reticular fibers had no correlation with either maturity or the actual site of ICH, and involved all the major intracranial arteries of that particular infant to the same extent. A normal pattern of reticular fibers could be found in some infants with ICH below 1500 gm birth weight, but all of the larger newborns with ICH had reticular fiber deficiency. Occasionally, a decrease of reticular fibers could also be observed in the arterial wall of infants without ICH, predominantly in babies with more than 1500 gm birth weight (Fig. 5).

Discussion

In premature infants, ICH generally develops in the periventricular germinal matrix and often ruptures into the ventricular system. Nevertheless, bleeding can also occur in other sites of premature brains, including the choroid plexus and the cerebellum. It can also be restricted to the subarachnoid space. 32,41

Despite the large amount of data based on both clinical and experimental observations, the exact pathogenesis of ICH has remained uncertain. Investigation has dealt almost exclusively with subependymal hemorrhage (SEH), and there is a general agreement that both physiological and morphological factors are responsible for the development of SEH in premature newborns. The physiological or systemic factors that have most frequently been considered in the pathogen-
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**FIG. 4.** Sections of basilar artery. Gomori's reticulin, × 400. **Left:** Specimen from a 2-day-old infant without intracranial hemorrhage (ICH), whose birth weight was 2100 gm, showing a normal pattern of reticular fibers. **Right:** Specimen from a 4-day-old infant with ICH, whose birth weight was 2400 gm. A paucity of reticular fibers is seen, predominantly in the outer portion of the media.

**FIG. 5.** Incidence of reticular fiber deficiency in the major intracranial arteries. I: without intracranial hemorrhage (ICH); II: with various types of ICH. Open bars represent the normal pattern of reticular fibers. Shaded bars represent cases with reticular fiber deficiency.

The richly vascularized germinal matrix has been regarded as the morphological basis of SEH. The matrix tissue at a younger gestational age is densely cellular and friable, not providing sufficient support to the blood vessels which are thin-walled and cannot be identified by light microscopy as arterioles, venules, or capillaries. However, electron microscopic investigation revealed that the capillaries of the germinal layer were structurally identical to those elsewhere in the immature brain; that is, they do not have a specific structure that could account for their frequent rupture at a younger gestational age. The exact site of rupture and the stages of the pathological process are still a matter of controversy. No sound data are available to explain the histological basis of hemorrhage arising elsewhere within the skull. The immaturity of the blood vessels is a vague concept and has not been supported by histological evidence.

A comparison of the major cerebral arteries of premature infants with and without ICH revealed differences in the thickness of the arterial wall and the amount of reticular fibers. One striking observation in infants with ICH was that the wall of the basilar artery was significantly thinner (suggesting hypoplasia) than that of infants of a similar gestational age and birth weight but without ICH. Of course, the possibility cannot be ruled out that the arteries of premature infants with ICH dilated before death due to loss of autoregulation contributing to the narrowness of the arterial wall. It is worthy of mention that there was no significant correlation between the thickness of the arterial wall and the actual origin of the ICH.

Another striking histological feature of the intracra-
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Intracranial major arteries of premature infants with ICH was the very frequent finding of reticular fiber deficiency. In the overwhelming majority of infants without ICH, the density of fine reticular fibers surrounding the individual smooth-muscle cells of the media strongly resembled that of individuals without vascular diseases, including mature newborn infants, children, and adults. The density of these fibers showed no change after the 26th gestational week. The intracranial arteries of most premature infants with ICH comprised disproportionately fewer reticular fibers in both regular and irregular distribution. If their distribution was irregular, the deficiency in reticular fibers was restricted mainly to the outer part of the media being similar to the pattern observed in the major intracranial arteries of adults with berry aneurysms.

It is important to emphasize that the internal elastic lamina of all premature infants has always been stained blue by trichrome, suggesting that the immature vessel wall does not contain true elastic elements but only the so-called "pseudoelastic fibers." These fibers are known to have the staining characteristics of both the collagen and elastic fibers. Lack of true elastic fibers appears to be the common feature of cerebral vessel walls of all premature infants. This was observed in 1854 but has generally not been considered. For this reason, the immature vessel wall is probably much less elastic than the arterial wall of children and young adults. Parenthetically, it is still unknown when elastin is first demonstrable in the internal elastic lamina of human arteries.

All forms of premature ICH are most probably the result of rupture of the small cerebral blood vessels. Since we found a significant relationship between the histological changes of the major intracranial arteries and the presence of ICH, we presume that these pathological changes involve the whole cerebrovascular system. The not infrequent coexistence of independent hemorrhages occurring in two different sites of the same brain appears to support this presumption. Moreover, Naeye found that the small extracranial arteries of newborns malnourished before birth generally contained fewer smooth-muscle cells, and that these individual cells had an abnormal cytoplasmic mass proving the existence of hypoplasia of small arteries. We may presume in all probability that hypoplasia and the partial lack of reticular fibers can considerably contribute to the weakness of the immature vessel wall, resulting in significant decrease of their resistance to hemodynamic stresses.

Our observations call attention not only to the pathological changes of the cerebral vasculature that might form the morphological basis of ICH, but also to the important role of physiological factors in its pathogenesis. Pathological alterations of the intracranial arteries cannot be found in every case of ICH and, conversely, ICH does not occur in every patient with pathological changes of the intracranial arteries. Below a birth weight of 1500 gm, the physiological factors seem to have a more dominant role, while in larger babies the structural alterations become more important for predisposing to ICH. On the basis of our previous observations, it seems reasonable to assume that infants who survive the postnatal period despite deficient reticular fibers in their arteries will become susceptible to developing intracranial berry aneurysms in a later period of their life.

The reason for hypoplasia of intracranial arteries and their lack of reticular fibers are unknown. Genetic and various other factors acting during early intrauterine life may be responsible. Further investigations are required to determine the factors that influence normal development of smooth-muscle cells and formation of reticular fibers in the media of the blood vessels. Confirmation is also needed that the changes observed in these infants do actually involve the whole vascular system.

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