Spontaneous germinal matrix and intraventricular hemorrhage in prematurely born rabbits

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Spontaneous hemorrhage into the ventricles in premature babies is a major problem, and neither its cause nor its pathogenesis is understood. A model is presented for the study of germinal matrix and intraventricular hemorrhage in the preterm rabbit. This animal is particularly suitable because, like the human, 1) the maximal growth of the brain occurs perinatally; 2) there is an abundant germinal matrix near term, and by birth this is substantially reduced; 3) there is no rete mirabile; 4) the blood flow to the brain is via internal carotid and vertebral arteries; 5) the maturation of the lungs is completed just before term; and 6) the rabbit pup can maintain a separate existence from the dam when delivered prematurely. Eight of 64 such animals were found to have developed spontaneous germinal matrix hemorrhage with or without rupture into the ventricles. Several physiological and chemical features characteristic of the premature rabbit are presented. The hemorrhage in the lagomorph might be a paradigm of that in infants, and its study may aid in the understanding of the pathogenesis of the process.

KEY WORDS • germinal matrix hemorrhage • intraventricular hemorrhage • prematurity • preterm rabbit

Bleeding into the ventricles of the brain in the newborn is a harsh complication of prematurity. It is said to cause or contribute to the death of 0.1% of live-born babies, and survivors of this event may suffer neurological, including intellectual, impairment and hydrocephalus. In our neurosurgical department, 40% of acquired forms of hydrocephalus were found to be due to neonatal intraventricular hemorrhage. The more serious hemorrhages seem now to be diminishing in frequency. Many causes have been proposed, including hypoxia, venous thrombosis, venous hypertension, alkali therapy, arterial hypertension (including impaired autoregulation of cerebral blood flow) enhanced fibrinolytic activity, and dehydration of the brain. The etiology remains uncertain, however, and the origin has been considered to be multifactorial.

Since treatment can only control intracranial pressure and hydrocephalus, it may maintain the current status, but cannot restore lost function or potential. Prevention of the bleeding is necessary, but it will be possible only when the pathogenesis is understood. A decade ago, the notion was prevalent that fatal intraventricular hemorrhage was, to paraphrase Osler on pneumonia in the elderly, "the premature baby's friend." This belief has proved incorrect in that a potential for good development has been found even in severely affected babies who would have been abandoned to their fate only a few years ago.

We have developed in the premature rabbit pup, delivered by caesarean section 3 to 5 days before term, a model of spontaneous germinal matrix and intraventricular hemorrhage. Such bleeding was encountered in eight of 64 animals studied. These little animals are thus shown to be susceptible to the disorder. We are contemplating subsequent investigation of a number of aspects of this study, including ultrastructural, metabolic, and behavioral factors, but this paper is confined to a description of the model.

Materials and Methods

The female rabbit ovulates only after copulation and, as a result, the gestational age of the litter can be determined from the date of mating. In this study, 19 female New Zealand White rabbits, weighing 3.0 to 4.5 kg and bred under visual observation (Day 0), were killed at 27 to 30 days gestation by rapidly injecting 30 ml of air into the marginal vein of the ear. The pups were delivered as quickly as possible by hysterotomy, aspirated to remove fluids from the pharynx, weighed, and placed in a brooder kept at 35°C. Total delivery time ranged from 7 to 15 minutes and was dependent on size of the litter.
Intraventricular hemorrhage in premature rabbits

In order to enhance and prolong survival during the first few hours of life, 0.2 ml of 10.8 mM glycerol solution was injected intraperitoneally immediately and at 4 and 8 hours after birth; this dosage does not affect serum osmolality. Glycerol, but not glucose, enhances survival of the preterm rabbit by serving as a substrate for energy metabolism. This substrate is required because of the inability of the premature rabbit to reverse glycogenolysis and initiate gluconeogenesis from lactate or alanine during the first hours of life. This impairment, together with low glycogen stores in the liver, which are 50% less than are found in full-term animals, puts these frail little animals at jeopardy early in life. It is not clear, however, that this is the only reason for enhanced survival following glycerol administration; in the preterm animal, glycerol is also converted rapidly to phosphatidylcholine, which acts to prevent respiratory insufficiency.

Of the 156 preterm animals delivered in the manner described, 4.5% failed to initiate spontaneous breathing or succumbed within the 1st hour of life. These were excluded from the study. The fact that the rest were able to initiate ventilation spontaneously is not surprising, since at 28 days of development the lungs of premature rabbits have been noted to be structurally mature and to have adequate amounts of surfactant in the pulmonary fluids.

The day after birth and once each day thereafter, surviving pups were weighed before and after receiving, either orally or by intragastric tubing, an amount of milk formula equivalent to 10% of the animals' body weight. For comparison, we studied 44 rabbits born vaginally to five dams at term (31 to 32 days).

Recordings of arterial, venous, and cerebrospinal fluid (CSF) pressures were successfully obtained from some premature rabbits delivered at 28 days gestation that survived for 1 day. These animals were anesthetized with tribromoethanol (90 mg/kg intraperitoneally). Polyethylene tubes, stretched to an appropriate diameter and filled with heparinized saline, were inserted into the femoral artery and vein of each animal. The animal was then turned over and placed in a sphinx-like position using both a block of polyester foam shaped to the contour of the animal's body and a small-animal stereotaxic apparatus. The blunt ear bars of the head holder were placed snugly just under the protruding ears of the animal to prevent lateral movement of the head. The snout clamp was used to angle the head appropriately and to prevent rostrocaudal movement. Once the animal was secured, a small-animal stereotaxic apparatus. The thin-walled, short-bevelled No. 25 to 27 needle was inserted into the cisterna magna percutaneously. The needle was connected to a fluid-filled polyethylene tube and in turn to a pressure transducer. Correct placement was determined by the appearance of respiratory fluctuations on the chart recording. Pressures were monitored with Statham strain-gauge transducers with zero reference set at the manubrium. Pressures were recorded on a multichannel direct-writing polygraph.

Blood samples obtained at the end of these experiments, either from the femoral artery or from the severed neck after decapitation, were used to determine Na⁺ and K⁺ concentrations by direct potentiometric analysis, osmolality by freezing point depression, and hematocrits. Water content of the brain was determined by desiccating whole, half, or sections of brain from selected animals to constant weight. Percent water content was calculated as follows: % water = (wet weight − dry weight) × 100/wet weight. Brain Na⁺ and K⁺ levels were determined in the same desiccated tissues by flame photometry.

The brains of 64 preterm and 17 full-term animals, which either died in the brooder or were killed following an anesthetic dose of pentobarbital (30 mg/kg), were removed from the cranium. The brains were then fixed by immersion in 10% buffered formalin and subsequently processed for histological study. Two to three 10-μ thick coronal sections were cut and stained with hematoxylin and eosin.

Results

At the time of delivery, most of the premature rabbits were able to breathe spontaneously. Surviving animals exhibited vigorous movements, appeared to be well oxygenated (as indicated by the pink appearance of their skin), and squealed when handled. Body weight of preterm animals at birth varied with gestational age (Fig. 1 left). Unlike full-term rabbits, which continued to gain weight after birth, premature animals lost weight, a trend that was reversed by the 3rd day of life. Hand feeding was successful in extending the survival time of the premature animal beyond the 24-hour period achieved with glycerol treatment alone. In the 28-day gestation age group, for instance, more than 60% of premature pups alive at 24 hours survived for 4 days (Fig. 1 right).

A representative recording of arterial, venous, and CSF pressures obtained from a premature rabbit 1 day after delivery at 28 days gestation is shown in Fig. 2, and average values for these pressures recorded

* Unilac milk formula from Upjohn Co., Kalamazoo, Michigan.
† Polyethylene tubes, PE 10, manufactured by Clay-Adams, Inc., Parsippany, New Jersey.
‡ Rat stereotaxic apparatus manufactured by Trent Wells, Tujunga, California.
* Potentiometer, Model Nova-1, manufactured by Nova Biochemical, Newton, Massachusetts.
† Freezing point osmometer, Osmette A, manufactured by Precision Systems, Sudbury, Massachusetts.
‡ Flame photometer, Model 343, manufactured by Instrumentation Laboratories, Watertown, Massachusetts.

J. Neurosurg. / Volume 56 / March, 1982
from 13 preterm pups are presented in Table 1. Although the systemic blood pressure is considerably lower than that reported for mature rabbits, it is comparable to that obtained from newborn rabbits.24 Average values for Na⁺ and K⁺ concentrations in blood and brain, for hematocrits, and for osmolalities are given in Table 2. Total brain water is similar to that (88.6%) reported for the fetal rabbit by Graves and Himwich.19 In comparison, the concentrations of Na⁺ and K⁺ in the brains of adult rabbits are (in mM/kg wet weight) in the 50's and 90's, respectively.24,49 The relatively high water and Na⁺ content associated with a low K⁺ content is, according to Katzman and Pappius,29 a reflection of the immaturity of the brain. The average serum potassium levels observed by us are higher than average values reported by Young,66 for fetal rabbits aged 23 to 31 days. On the other hand, our values for sodium were lower (133.2 versus 142.0 mM/kg wet weight). Nevertheless, our values are comparable to those reported by others and cited by Kozma, et al.33 in their review article.

Grossly, with the exception of the longitudinal fissure, the dorsal surface of the perinatal rabbit brain is devoid of topographical landmarks. The brains from premature rabbits without hemorrhage were usually pale and somewhat translucent in appearance. The lack of blood within surface and cortical blood vessels made the cortex appear avascular. Coronal sections revealed slit-like lateral ventricles containing choroid plexus, the vessels of which were filled with blood.

In contrast, in premature rabbits with hemorrhage, pooled blood was occasionally seen within the cranial subarachnoid spaces. In animals with massive hemorrhages, blood within the ventricles could easily be discerned through the translucent cortical mantle. Coronal sections of these brains revealed dilated ventricles filled with blood, and hemorrhages within the germinal matrix areas.

Microscopic examination of brains from preterm and term rabbits revealed that the extensive subependymal germinal matrix present in 27- to 28-day-old preterm pups (Fig. 3B) had diminished considerably by term. In the 28-day-old preterm pup, the germinal matrix was most prominent on the borders of the anterior horn of the lateral ventricle. In this area, it appeared densely cellular and richly vascularized. The matrix bordering the body and inferior horn of the ventricles was considerably thinned out and less prominent.

Eight (12.5%) of the 64 brains obtained from premature rabbits revealed minimal (Fig. 3D) to extensive bleeding in the germinal matrix (Fig. 3A and B). Five of the brains exhibited extensive germinal matrix hemorrhages which, as indicated by the appearance of blood within the ventricles, had erupted through
Intraventricular hemorrhage in premature rabbits

**TABLE 1**

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<th>Physiological measurements from premature rabbits</th>
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<tr>
<td><strong>Factor</strong></td>
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<tr>
<td>age (days)</td>
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<tr>
<td>body weight (gm)</td>
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<tr>
<td>brain weight (gm)</td>
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<tr>
<td>arterial pressures</td>
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<tr>
<td>venous pressures</td>
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<td>cerebrospinal fluid pressures</td>
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<tr>
<td>heart rate (min⁻¹)</td>
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<td>respiratory rate (min⁻¹)</td>
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**TABLE 2**

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<th>Chemical measurements in blood and brain of premature rabbits</th>
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<tr>
<td><strong>Factor</strong></td>
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<tr>
<td>brain Na⁺ (mM/kg wet wt)</td>
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<tr>
<td>brain K⁺ (mM/kg wet wt)</td>
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<tr>
<td>brain H₂O (%)</td>
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<tr>
<td>blood Na⁺ (mM/liter)</td>
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<tr>
<td>blood K⁺ (mM/liter)</td>
</tr>
<tr>
<td>hematocrit (%)</td>
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<td>osmolality (mOs/kg water)</td>
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the wall of the ventricle. Although the extensive hemorrhages were limited to the germinal matrix areas, small petechial hemorrhages were observed in other areas (Fig. 3D). Blood within the lateral ventricles was also observed to originate from engorged choroid plexus (Fig. 3C) in two animals.

No hemorrhages were observed in the 17 brains examined from term animals.

**Discussion**

The incidence of subependymal germinal matrix or ventricular hemorrhage in the preterm rabbit reported here (12.5%) is somewhat less than the incidence observed (approximately 40%) for the premature infant at risk.¹,¹¹,¹⁴ Obvious reasons, including the mode of delivery, clinical status, or species difference, may account for this discrepancy. Just as likely is the possibility that our histological survey, which was limited to two to three coronal sections per brain, did not uncover all the germinal matrix and ventricular hemorrhages that occurred in the premature rabbits. Nevertheless, the macroscopic and microscopic appearance of the hemorrhage in the preterm rabbit was similar to that observed in premature infants with intraventricular hemorrhage. In addition to hemorrhage in the germinal layer, often from multiple sites, ventricular hemorrhage from choroid plexus was seen and petechiae were found in the parenchyma in some animals. A similar distribution of hemorrhages was observed in the study of the process in fetal sheep by Reynolds, et al.,⁴² and in beagle pups by Goddard, et al.¹⁶,¹⁷ In infants, the concurrence of cerebellar hemorrhage has been reported.²²,²⁷

Several animal studies relating to intracranial hemorrhage in the newborn have been made. We may dismiss as not pertinent to the present discussion the experiments in older cats by Turbeville, et al.,⁴⁸ and the study in which fetal guinea pigs near term, successfully resuscitated after intrauterine asphyxiation, developed intracranial hemorrhage (these latter hemorrhages were later considered by the author to have been agonal.⁴⁴) Experiments similar to the latter were carried out in rabbit fetuses by Takashima and Tanaka,⁴⁵ who found subependymal but not ventricular hemorrhages, particularly in those made hypoxic in utero at 28 to 30 days gestation and killed 5 minutes after delivery.

Because intraventricular hemorrhage occurs after birth in the premature infant, it would seem that use of surviving preterm animals would constitute a more exact model, but unfortunately preterm animals generally do not live beyond a few hours after birth. Reynolds, et al.,⁴² have circumvented this problem by using the exteriorized fetal lamb which, at 58 to 85 days of gestation (term, 151 days), has a subependymal germinal matrix similar to that of the human infant. A combination of asphyxia and intermittent increases in arterial or venous pressure led to hemorrhages in the germinal matrix and ventricles. The experiments suggested that similar mechanisms may be involved in the production of intraventricular hemorrhage in the newborn infant. But in a recent clinical

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**Fig. 2.** Representative recording of cerebrospinal fluid (CSF), venous, and arterial pressures from a 1-day-old premature rabbit delivered at 28 days gestation.
FIG. 3. A: Macroscopic appearance of intraventricular hemorrhage in the brain of a rabbit delivered at 28 days gestation and surviving 24 hours. Bar = 1 mm. B: The microscopic appearance of a germinal matrix and intraventricular hemorrhage (arrows) in a premature rabbit delivered at 28 days gestation and surviving 2 days. Bar = 100 μ. C: Hemorrhage (black arrows) arising from a break in the choroid plexus (open arrow) of the lateral ventricle in a rabbit pup delivered at 28 days and surviving 2 hours. Bar = 100 μ. D: Widespread hemorrhages (arrows) appearing in the germinal matrix and white matter of a preterm pup delivered at 28 days gestation and surviving 2 hours. There is blood within the ventricle. Bar = 100 μ.

study, in which blood pressure was continually monitored from birth, mean arterial pressure during the first 6 hours after birth in babies who died with intraventricular hemorrhage was lower, as was the severity of asphyxia, than in surviving babies with low birth weight who served as matched controls. Indeed, in three of the nonsurviving infants, an acute episode of hypotension preceded the intraventricular hemorrhage.

As indicated by Pape and Wigglesworth, the response of the cerebral vasculature and flow to the imposed stress may not be comparable in sheep to that in the human infant. The exteriorized fetal sheep is still connected via the umbilical cord to the dam and therefore is not exposed to the stress faced by the premature infant which is called upon to thrive in an unexpected and different environment during a time of development which normally occurs in utero.

Recently, Goddard, et al., were able to produce subependymal and ventricular hemorrhage in term beagle puppies either by the induction of hypertension following the administration of phenylephrine hydrochloride or by the induction of hypercarbia. Increased blood pressure was not a feature of children who bled, as compared with controls in the study of Goldberg, et al., but there was a significant volume overload in the former, leading, in the authors’ view, to increased venous pressure.

The inherent difficulties associated with comparative studies of brain development are well recog-
Intraventricular hemorrhage in premature rabbits

Intraventricular hemorrhage in premature rabbits 1-7,28 Not only are there great differences in gross and microscopic anatomy among species, but also there are functional, biochemical, and developmental variations that must be considered. On a practical basis, the magnitude and complexity of the problem becomes apparent when one considers that chronologically pre- and postnatal brain development in the human is telescoped into a fraction of the time in the more common laboratory animal. Obviously, there is no readily available species that can provide a maturation process of brain development that compares with that of the human. However, one index of brain development which has been found useful in assessing perinatal stress on a comparative basis has been "the brain growth spurt period."9 This phase of central nervous system development represents a period when the rate of brain growth is maximal and the brain is said to be highly vulnerable to perinatal stress. Its occurrence relative to birth has been used to classify species as pre-, peri-, or postnatal brain developers. But even under these conditions, applicability to the human condition of results obtained from animal studies during this phase of brain development can be expected to be critically dependent on the relative timing, intensity, and duration of the adverse stimulus.

For this, as well as other reasons, Pape and Wigglesworth argued in their monograph on intraventricular hemorrhage 3 that investigators of this problem should attempt to use those species that provide the most comparable brain and lung development to the human infant as well as a central nervous system vasculature that is similar to that of man.

The rabbit, like the human, is classified as a perinatal developer 2,9,10 and, at 27 to 30 days gestation, exhibits a substantial subependymal germinal matrix as shown in this report and earlier by Fernández. 15 Moreover, unlike other laboratory species, it lacks a carotid rete mirabile, the presence of which in species such as the sheep and dog may confound direct comparison to the human of relationships between carotid and cerebral flow dynamics. Furthermore, blood flows to the brain of the rabbit primarily via the internal carotid and vertebral arteries; 7,28 and, since the proportion of brain perfused by each of these vessels is similar to that in the human, 9 changes in cerebral vascular dynamics in response to cardiovascular fluctuations probably resemble those observed in the human. Finally, the ability of the 28-day-old preterm rabbit to maintain a separate existence out of the uterus testifies to the morphological and biochemical maturity of the animal's lungs at this age. The present work also shows that, although these animals are quite small, monitoring of several physiological variables can be carried out.

A measure of maturity of the brain is the water and electrolyte pattern, which changes with development from a watery to a drier tissue, and from a high sodium-low potassium pattern to the opposite. 8,9 In comparison to the brain of the newborn infant, 8,9 that of the rabbit is slightly less moist and has a transitional sodium-potassium ratio approximating 1 (Table 2).

Finally, intraventricular hemorrhage is a function of prematurity and the state of development of the brain, and in those respects the present model, unlike others, seems to resemble the human situation.

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References


J. Neurosurg. / Volume 56 / March, 1982


A. V. Lorenzo, K. Welch and S. Conner

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