Acta Neuropathol (Berl) (1987) 72:268–276

Acta Neuropathologica


# Morphological aspects of the development of hydrocephalus in a mouse mutant (SUMS/NP)*

**H. C. Jones, S. Dack, and C. Ellis**
Department of Zoology, University of Hull, HU6 7RX, UK

**Summary.** The SUMS/NP is a mouse mutant with a recessive gene for congenital hydrocephalus. The condition is detectable outwardly at 3 days after birth and affected animals die soon after weaning. The heads of fetuses from 14 to 20 days gestation and at 1, 4, 5, 12 and 18 days after birth have been sectioned for light microscopy and the volume of the lateral ventricles measured in all but the three oldest ages. At 16 days gestation and earlier all fetuses had a relatively large lateral ventricle volume. Hydrocephalus was first detected from volume measurements at 18 days gestation and generally became progressively more severe with age. Hydrocephalic animals, in addition to lateral and third ventricle dilatation, always showed a reduction in the cross-sectional area of the cerebral aqueduct or a total absence of the aqueduct. All hydrocephalics, with the exception of two fetuses, also had cystic cavitation of the forebrain around the lateral ventricles. Electron microscopy of animals with a reduced aqueduct showed the ventral part to be absent in hydrocephalics.

**Key words:** Mouse mutant – Congenital hydrocephalus – Lateral ventricle volume – Cerebral aqueduct

The value of animal models is well recognised in the investigation of human disease and many studies on hydrocephalus have been carried out using animals (Hochwald 1985). Hydrocephalus occurs most frequently in the perinatal period in man, thus animals showing congenital hydrocephalus are particularly appropriate for the study of the development of the disease process. In mice, congenital hydrocephalus has arisen several times from spontaneous mutation; e.g. hydrocephalus-3 (Grüneberg 1943), obstructive hydrocephalus (Borit and Sidman 1972), hydrocephalic polydactyl (Bryan et al. 1977) and congenital hydrocephalus (Grüneberg and Wickramaratne 1974). We describe here the morphological aspects of the development of congenital hydrocephalus in a further inbred strain of mice (SUMS/NP) which has a recessive gene for hydrocephalus. Preliminary reports of this mutant have appeared elsewhere (Punt et al. 1982; Jones 1984).

## Materials and methods

The SUMS/NP mouse is a hydrocephalic mutant which originated from Guy's Hospital, London. The mice have a presumed autosomal recessive gene for hydrocephalus which affects, on average, 13% of the offspring from heterozygous parents. The hydrocephalus is detectable outwardly by 3–4 days after birth and affected animals die soon after weaning.

*Light microscopy: heads.* The heads of three complete litters of fetuses from each of the following gestational age groups: 14–16 days, 17–18 days and 19–20 days, were fixed in 2% paraformaldehyde, 2.5% glutaraldehyde and 0.1 M phosphate buffer at pH 7.4 and prepared for conventional wax histology. Transverse sections, 6 μm thick, of the brain plus surrounding meninges and skull were cut and stained with Mallory's triple stain. In addition, two complete 1-day-old litters taken from known heterozygous parents and both normal and hydrocephalic neonates at 4, 5, 12 and 18 days were processed as for fetuses.

*Light microscopy: spinal cords.* Spinal cords from normal and hydrocephalic mice 8 and 14 days old perfused intravascularly as above, were embedded for wax histology and a few sections were collected every 1 mm throughout the length of the spinal cord for examination of the central canal.

*Electron microscopy: cerebral aqueduct.* Mice from two age groups, 1–3 days (two normal and five hydrocephalic) and 6–8 days (two normal and three hydrocephalic), were perfused

*Offprint requests to:* H. C. Jones (address see above)
* Supported by the Wellcome Trust, and Action Research, The National Fund for Research into Crippling Diseases

intravascularly with the same fixative. The presence or absence of enlarged lateral ventricles was determined by dissection before processing and embedding the region containing the aqueduct in Emix resin. Semithin transverse sections were stained for light microscopy with toluidine blue. Ultrathin sections were taken from selected regions of the narrowest part of the aqueduct, stained in uranyl acetate and lead citrate and viewed in a Jeol 100C electron microscope. In two additional 2-day-old mice (one normal and one hydrocephalic) the complete length of the aqueduct was sectioned for light microscopy only.

*Volume and area measurements.* Stained wax sections were projected on to a graphics tablet attached to an Apple II microcomputer and the regions to be measured were outlined with the tablet pen. Lateral ventricle and forebrain (telencephalon plus diencephalon) volumes were calculated for fetuses and 1-day neonates and for one 4-day hydrocephalic and the volume and surface area of the two lateral ventricle choroid plexuses were measured for 1-day-old neonates.

*Cerebrospinal fluid flow pathways.* Neonatal mice from three age groups, 1–2 days, 4–8 days and 10–21 days, were anaesthetised and a glass micropipette placed in one lateral ventricle for infusion as described previously (Jones 1985). Between 1 and 5 µl fluorescein-labelled dextran (mol. wt. 150,000; 100 mg/ml in artificial cerebrospinal fluid) was infused into one lateral ventricle. Between 10 and 20 min after the start of the infusion the mice were frozen whole in liquid nitrogen and the surface of the sectioned heads examined and photographed under a UV lamp for the distribution of fluorescence.

## Results

### *Histology*

*Normal brain development in fetuses.* During the period studied, 14 to 20 days gestation, there was considerable maturation of the brain and ventricular system. At 14 days gestation, only the fourth ventricle choroid plexus was present, whereas by 20 days gestation all three plexuses were well developed. In fetuses, as in the adult mouse, the cerebral aqueduct connects the dorsal third ventricle to the mesencephalic ventricle (also known as the posterior collicular recess). In fetuses up to 17 days gestation, however, the ventral part of the third ventricle also connected with the mesencephalic ventricle thus providing an alternative ventral route for the passage of fluid out of the third ventricle. Subsequently this ventral connection disappeared leaving the aqueduct as the sole route to the mesencephalic ventricle in animals over 18 days gestation. More caudally, the mesencephalic ventricle opened directly into the fourth ventricle. The lateral foramina of Luschka in the recesses of the fourth ventricle were closed in fetal mice but were open by one day after birth. Between 14 and 20 days gestation, the lateral ventricles decreased from 13.4% to 1.0% of the forebrain volume (Figs. 1, 2). This was due to 5-fold decrease in lateral ventricle volume and a 4-fold increase in forebrain volume.

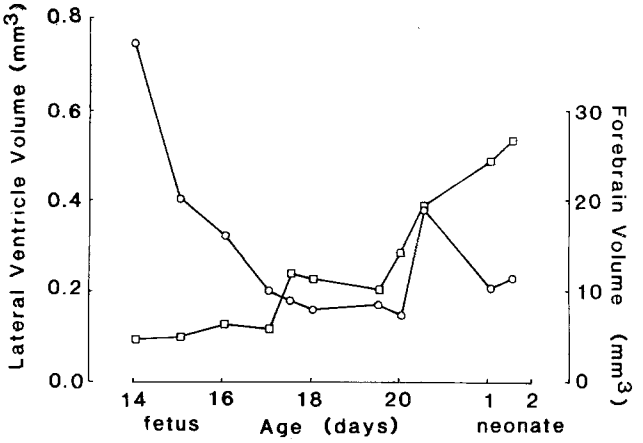


**Fig. 1.** A plot of lateral ventricle volume (○) and forebrain volume (□) against age for normal fetuses and neonates. Values are means, $n = 4$ to 14

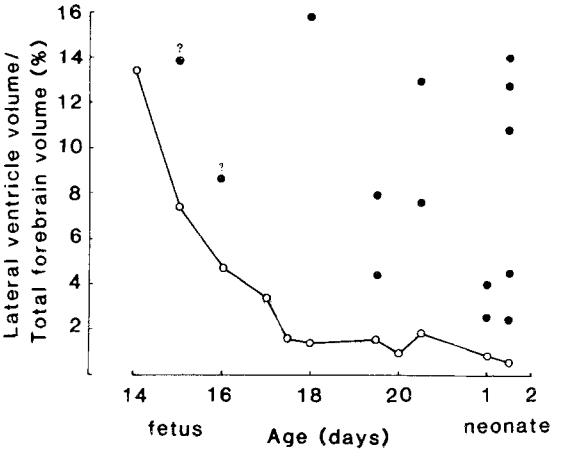


**Fig. 2.** A plot of lateral ventricle volume expressed as a percentage of total forebrain volume (○) against age for normal animals. Values are means, $n = 4$ to 14. The hydrocephalic individuals (●) have been included as single points together with two uncertain individuals (●?)

*Hydrocephalic fetuses.* Five fetuses aged from 18 to 20 days gestation were found to be hydrocephalic with a lateral ventricle volume larger than the remaining fetuses in the litters (Figs. 2, 3, 4a, b). Four of these had ventricular volumes larger than the mean volume obtained for the youngest fetuses at 14 days gestation (0.74 $mm^3$) and the fifth was close at 0.62 $mm^3$. These fetuses had enlarged third ventricles also. Of the five hydrocephalic fetuses, three showed a small degree of cystic cavitation of the forebrain around the lateral ventricles either unilaterally (two fetuses) or bilaterally (one fetus). The cerebral aqueduct in two of the

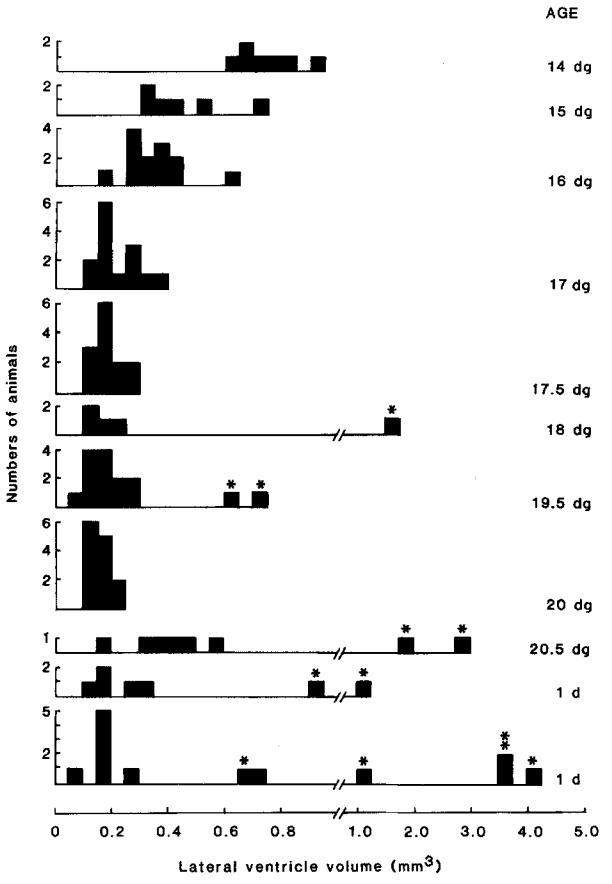


**Fig. 3.** Frequency distribution for total lateral ventricle volume for individual animals from 11 litters aged between 14 days gestation and 1 day after birth. Definite hydrocephalic individuals have volumes larger than the majority from the same the litter and are labelled (asterisk)

hydrocephalics was reduced, such that an aqueduct normally elongated in the dorsoventral plane (Fig. 4c) was seen as a circular or oval group of ependymal cells which in some places had a small lumen. This reduced, and dorsally situated aqueduct, extended in the rostral-caudal direction for up to 700 μm. In the other three hydrocephalic fetuses the dorsal aqueduct was absent for between 300 and 700 μm, such that there were no ependymal cells or lumen (Fig. 4d). These three fetuses had extremely dilated third ventricles (Fig. 4b). In addition, a few fetuses with apparently normal ventricle volumes for their age also had an aqueduct reduced in area. Prior to 18 days gestation it was not possible to determine with certainty whether the fetuses were hydrocephalic. For the five hydrocephalic fetuses, although the combined lateral ventricle volume was much higher than normal, the volume of the forebrain alone (excluding ventricles) was not less than normal suggesting that ventricular expansion has not occurred at the expense of the brain.

*Hydrocephalic neonates.* Histology was carried out on neonates at 1, 4, 5, 12 and 18 days. Ventricle volumes were measured only in 1-day neonates and one 4-day hydrocephalic. At 1 day the ventricle volumes of normal mice expressed as a percentage of total forebrain volume were slightly lower than for the late fetuses (Figs. 1, 2). Among animals from the two 1-day-old litters sectioned (20 mice in all), eight were classed as hydrocephalic using ventricle volume as the criterion (Figs. 2, 3). One hydrocephalic animal had unilateral ventricular dilatation only and all except this one also had an enlarged third ventricle. Seven of the eight had cystic cavitation around the lateral ventricles, either unilaterally of bilaterally. In six of these, the aqueduct was reduced to a small dorsal group of ependymal cells, either with or without a visible lumen, and in one the dorsal aqueduct was absent for around 600 μm. Another had a large ventricle volume but no cystic cavitation or abnormal aqueduct. In addition, in one apparently normal animal the aqueduct was reduced in area. As in the fetuses, the brain volume in neonatal hydrocephalics was not significantly reduced when compared to normals.

After birth the hydrocephalus progresses rapidly so that by 4 or 5 days there is a large increase in the lateral ventricle volume and in the cystic cavitation around the ventricles. In the one 4-day animal the ventricle volume was 24% of the forebrain and the total volume of ventricles plus cystic cavitation was 37%. By 4 days there was a marked reduction in cortical thickness with a corresponding increase in cavitation and by 12 and 18 days the cortex was only a few cells thick and ventricular ependyma was absent in some regions. The fourth ventricle and the lateral foramina of Luschka appeared normal in all hydrocephalic animals.

The volume and surface area of the combined lateral ventricle choroid plexuses measured at 1 day was found to be $0.089 \pm 0.003$ mm$^3$ (mean $\pm$ SEM) and $4.84 \pm 0.16$ mm$^2$ respectively for six normal mice and $0.066 \pm 0.002$ mm$^3$ and $4.11 \pm 0.091$ mm$^2$, respectively for five hydrocephalic mice from the same litter. These differences were significant, $P < 0.001$ for volume, and $P < 0.01$ for area.

The central canal of the spinal cord showed no differences between the normal and hydrocephalic mice at either age group studied.

*Electron microscopy of the cerebral aqueduct.* Electron microscopy was carried out on neonatal mice where the presence of hydrocephalus could be determined with certainty from the dilatation of the lateral ventricles observed during trimming after the initial fixation. Apart from one with a partially absent aque-

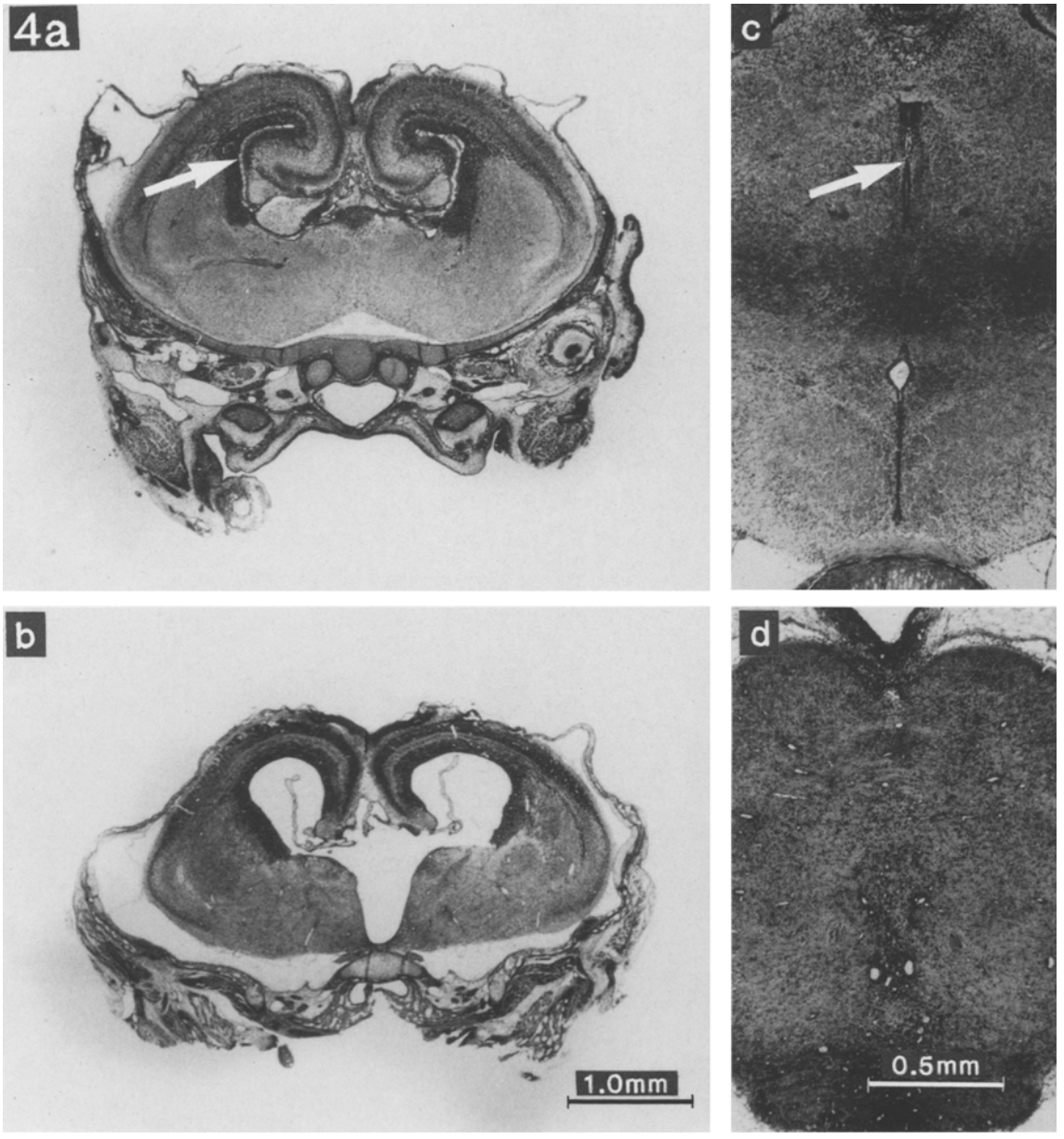


**Fig. 4a–d.** Light micrograph of transverse sections through the brains of two 18-day gestation fetuses. **a** Forebrain section of a normal fetus showing slit-like ventricles (*arrow*). **b** Forebrain section of a hydrocephalic fetus showing enlarged lateral and third ventricles. **c** Section through the aqueduct region of the same fetus as in **a**. The normal aqueduct (*arrow*) is elongated in the dorsoventral plane. Below it there is an extension of the ventral third ventricle which by 18 days gestation, is closed without connecting to the more caudal parts of the ventricular system. **d** Section through the aqueduct region of the same hydrocephalic fetus as in **b** showing a complete absence of aqueduct ependyma and lumen. Wax sections, **a** and **b** at the same magnification; **c** and **d** at the same magnification

duct, all hydrocephalics had reduced aqueducts (Fig. 5a–c) which continued in a similar way for up to 850 μm caudally, before opening into the mesencephalic ventricle. In contrast to the appearance in wax sections, the superior resolution of resin sections showed that where an entire layer of ependymal cells was present in hydrocephalics, there was always a small lumen. This lumen was about the same width as that in normals, 5–10 μm (Fig. 5b, c). In addition, in one 8-day hydrocephalic, the dorsal aqueduct was absent for the initial 450 μm and then present with a very small lumen for a further 880 μm. The reduced aqueducts in hydrocephalics were surrounded by ependymal cells which were indistinguishable from aqueduct ependymal cells of normal animals (Figs. 6, 7). The ependymal cells were

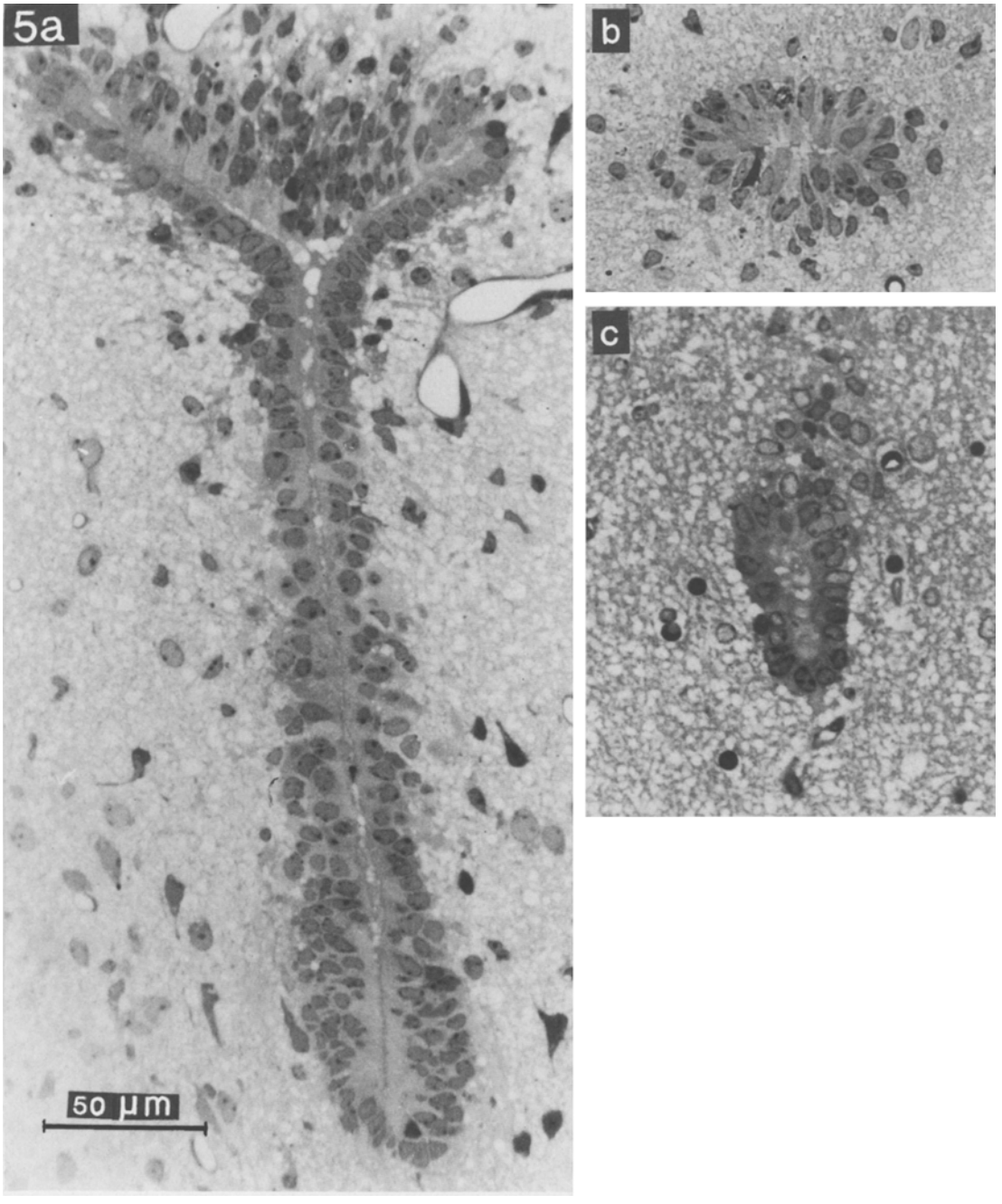


**Fig. 5.** Light micrographs of semithin transverse resin sections though the aqueduct of **a** a normal 2-day-old mouse, **b** a 2-day hydrocephalic and **c** an 8-day hydrocephalic. In the hydrocephalics the aqueduct is reduced from an elongated 'Y' shape to a small oval lumen surrounded by ependymal cells. Toluidine blue stain, all micrographs at the same magnification and orientated with the dorsal part upwards

columnar in shape with apparently normal cytoplasmic organelles and clearly defined apical intercellular gap junctions and zonulae adherentes. On the luminal surface there were numerous cilia and microvilli and the lumen contained deposits of amorphous material (Fig. 6) which was also seen in normal aqueducts. There was no evidence for subependymal oedema or widening of the intercellular clefts. In some animals the lumen contained macrophage-like cells (Fig. 7), but these were infrequent and may not have any particular significance for hydrocephalus. In two animals some of the ependyma surrounding the aqueduct was absent and there was apparent invasion of the lumen by neural and/or glial

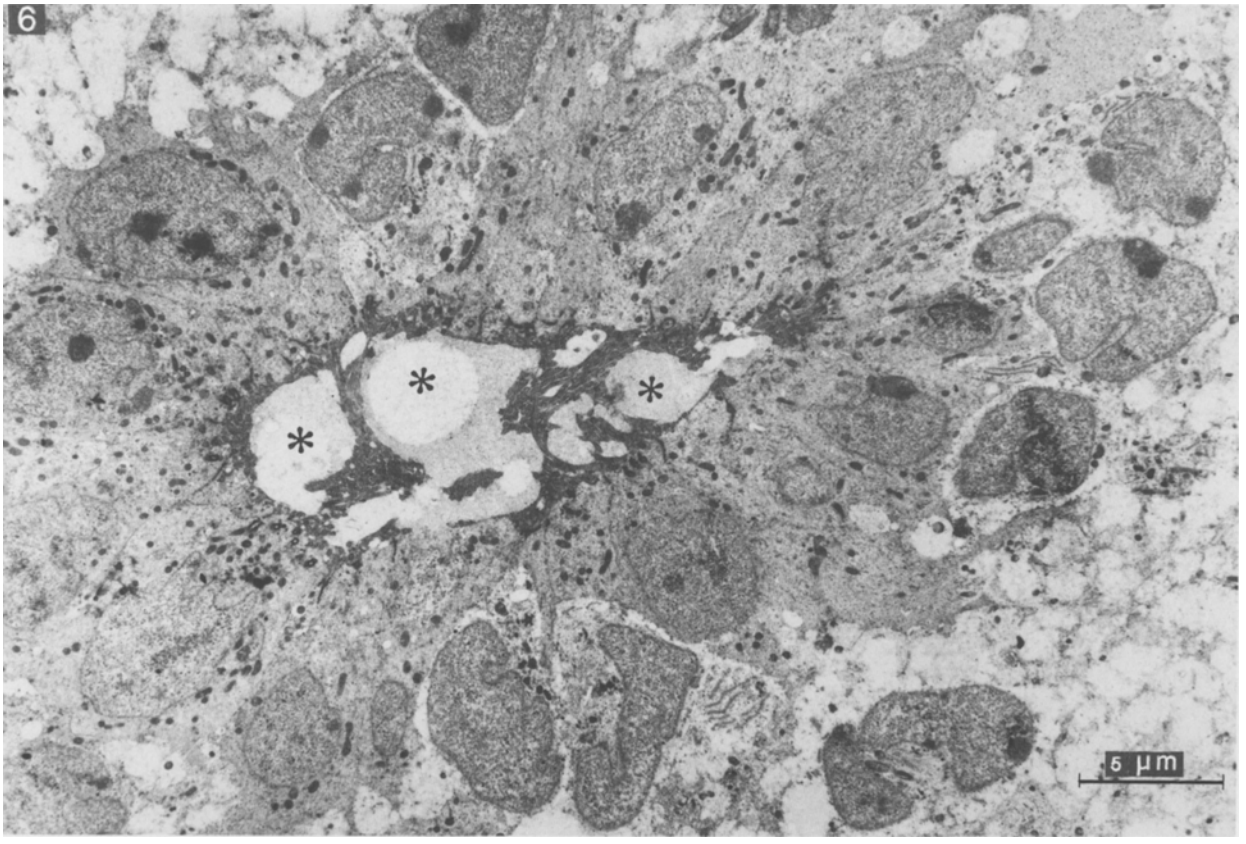


**Fig. 6.** Electron micrograph of the complete aqueduct from a 2-day hydrocephalic mouse. The oval-shaped lumen (*asterisks*) is surrounded by ependymal cells which appear similar to those of normal mice

processes which, intermingled with ependymal cilia, and completely filled the lumen (Fig. 7). In these cases, apart from the presence of cilia, the lumen was no different in appearance to the surrounding developing brain.

*Infusion experiments.* Infusion of fluorescein-labelled dextran into the lateral ventricle of normal neonates resulted in spread of fluorescence into the third, mesencephalic and fourth ventricles, the subarachnoid space around the hindbrain and, in some animals, into the spinal cord and the mesencephalic subarachnoid space. Among the hydrocephalics infused at 1 or 2 days, six had a normal distribution but five had only very limited amounts of fluorescence caudal to the third ventricle. Among hydrocephalics aged 4 to 8 days after birth, four had a normal distribution but a further 12 animals had no spread of fluorescence caudal to the third ventricle. Between 10 and 21 days all hydrocephalics had fluorescence confined to the lateral and third ventricles with or without slight spread into the subarachnoid space overlying the brain. This was thought to have left the ventricles via a ventriculostomy because the cortical mantle becomes extremely thin and probably breaks down at this time.

## Discussion

Over the developmental period 14 days gestation to 1 day after birth, there was a steady increase in forebrain volume of around fivefold, indicating that this is a period of rapid growth for the mouse brain. During this period, the combined lateral ventricles decreased from 13% to around 1% of the total forebrain volume. The normally large lateral ventricles at 14 days gestation make hydrocephalus due to lateral ventricle dilatation more difficult to detect in the younger fetuses. The same period, from 14 days gestation, covers the development of the subarachnoid space in the mouse (McClone and Bondareff 1975). Furthermore, the development of the choroid plexuses and the observation that microscopical interependymal pores occur in the roof of the rhombencephalon from 14 days gestation onwards (Jones et al., in press), suggest that fluid secretion starts during this period.

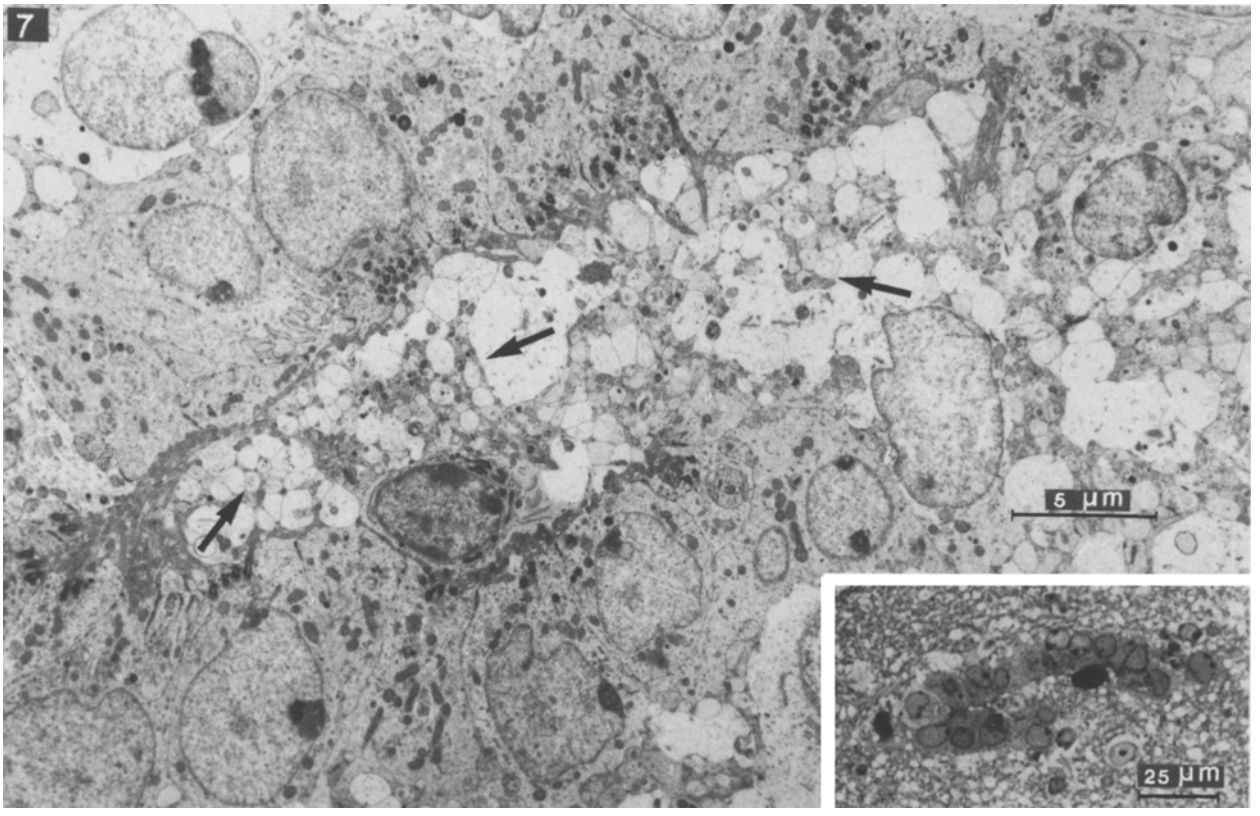


**Fig. 7.** Electron micrograph of the aqueduct from a 6-day hydrocephalic mouse. On the right hand side some ependymal cells are absent and there is apparent invasion of the lumen by brain cell processes (*arrows*) similar to those in the surrounding brain. The dark cell in the lumen may be a macrophage. *Inset* is a light micrograph of the same area from an adjacent section

In man, hydrocephalus is thought to arise in one of three ways: through an overproduction of cerebrospinal fluid (CSF), through an obstruction of the normal fluid flow pathway or through a defect in the CSF absorption mechanism. In many cases, however, it is not possible to determine with certainty the primary cause of hydrocephalus in man and this is particularly true for congenital hydrocephalus where a developmental abnormality may be involved. It is also true that the pathogenesis of congenital hydrocephalus in rodents is poorly understood. This investigation, the first to study fetal ages in mouse, has shown that the SUMS/NP mouse develops hydrocephalus in late gestation, although it is not detectable outwardly until 3–4 days after birth, by which time the hydrocephalus is quite severe and death occurs at around 3 weeks of age. Superfically, the hydrocephalus in SUMS/NP resembles that seen in other strains of mutant mice such as hydrocephalus-3 (hy-3, Grüneberg 1943; Berry 1961; Raimondi et al. 1973), obstructive hydrocephalus (oh, Borit and Sidman 1972) and hydrocephalic-polydactyl (hpy, Bryan et al. 1977) in that the sequence of pathogenesis is similar. None of these strains has been investigated prenatally and therefore it is not possible to be certain when the onset of ventricular dilatation occurs. The only existing strain of mouse with prenatal hydrocephalus is congenital hydrocephalus (ch) but this strain has extremely severe and totally different pathological manifestations (Grüneberg and Wickramaratne 1974).

There is one major feature of the hydrocephalus seen in SUMS/NP which is not seen in other strains and that is the occurrence of a reduced or absent aqueduct in the early stages of the disease process. In hy-3 and oh the aqueduct closes in the later stages of hydrocephalus and is thought to be a process which occurs secondarily to the ventricular dilation (Raimondi et al. 1976; Borit and Sidman 1972). In the results reported here all animals which could be identified with certainty as hydrocephalic had aqueducts which were either very much reduced in cross-sectional area or completely absent for part of their length. Thus it seems likely that an abnormality of the aqueduct may be the cause of hydrocephalus in this mutant strain. This is supported by recent experi-

ments on postnatal SUMS/NP, which measured the resistance to drainage of the CSF during the development of the hydrocephalus (Jones 1985). In hydrocephalics, the resistance to drainage from the lateral ventricles was higher than for normal animals of the same age, whereas the resistance to drainage from the cisterna magna was not increased in hydrocephalics. This shows that hydrocephalics have a reduced drainage of fluid out of the ventricular system, without any defect in the drainage of CSF from the subarachnoid space. It does not seem likely that the hydrocephalus in SUMS/NP occurs through the overproduction of CSF because the choroid plexuses were not enlarged in 1-day hydrocephalics.

The suggestion that in SUMS/NP the hydrocephalus is caused by a reduction in the size of the aqueduct is further supported by the timing of its appearance at 18 days gestation, since an abnormal aqueduct would not have any significant effect on CSF flow prior to 18 days gestation because of the alternative ventral pathway. The possibility cannot be completely excluded, however, that some less obvious change in the ventricular system may be the primary cause of hydrocephalus. If, as seems likely, an abnormal aqueduct is the cause of the hydrocephalus, the results show that there is not a complete obstruction in all cases. This was apparent in resin-embedded tissue, where a small lumen was present throughout the aqueduct in all animals except those where no aqueduct ependyma was present and the infusion experiments support this. It is possible that the hydrocephalus may be caused by an insufficient aqueduct which cannot conduct fluid at the required rate. This is supported by experiments on fixed human aqueducts, which showed that the aqueduct is only just large enough to pass the CSF normally vented during systole (White et al. 1979).

In SUMS/NP mouse there is a variable manifestation of the aqueduct abnormality from reduction in area to complete absence of aqueduct. In general, animals without an aqueduct tended to have larger lateral ventricles than with those only reduced aqueducts but this was not true in all cases and those with reduced aqueducts certainly had clearly defined hydrocephalus. In addition, some other animals with normal ventricles had reduced aqueducts, as seen in wax section histology. Whether or not these animals would have progressed to hydrocephalus had they survived to a later stage, cannot be determined.

Much has been written about aqueduct stenosis as a possible cause of hydrocephalus in man, since this is a feature frequently observed at autopsy (Russell 1949) and at least one type of hydrocephalus has been attributed to it (Bickers and Adams 1949). Because of a shortage of early pathological material, there is no good evidence that hydrocephalus is actually caused by an abnormal aqueduct in man and it has been convincingly argued that all aqueduct stenosis is secondary to the hydrocephalus (Williams 1982).

In animals, only one example of primary closure of the aqueduct causing hydrocephalus has been described, that caused by the teratogenic effects of cuprizone on weanling mice (Kesterson and Carlton 1970). In this example, oedema of the mesencephalon preceded the aqueduct stenosis and these authors describe a loss of ependyma from the walls of the aqueduct resulting in fusion of the subependymal surfaces. There have been a number of electron microscopy studies on rodent hydrocephalus but many have concentrated on changes in the walls of the lateral ventricles (e.g. Borit and Sidman 1972). One study on the aqueduct in the hy-3 mouse with secondary aqueduct occlusion showed that the aqueduct becomes compressed by the subependymal oedema, resulting in closure of the lumen (Raimondi et al. 1976). In this case the ependyma had a normal columnar appearance but it was stretched and torn in places where there was surrounding oedema. In the hamster with reovirus-induced hydrocephalus, the aqueduct lumen became filled with detached ependymal cells and macrophages as a consequence of the inflammatory process (Nielsen and Baringer 1972). In this study, there was no evidence for oedema or inflammation as contributory factors and we can only speculate on the mechanism by which the aqueduct becomes reduced or lost in SUMS/NP. It is possible that, since the remaining aqueduct ependymal cells appear normal, there is some abnormal growth of one or more other brain cellular components which causes a loss of aqueduct ependyma during development. The observation that in two animals examined by electron microscopy, there was invasion of the lumen by brain cell processes where some periaqueduct ependymal cells were absent, lends support to this suggestion.

## References

Berry RJ (1961) The inheritance and pathogenesis of hydrocephalus-3 in the mouse. J Pathol Bacteriol 81:157–167

Bickers DS, Adams RD (1949) Hereditary stenosis of the aqueduct of Sylvius as a cause of congenital hydrocephalus. Brain 72:246–262

Borit A, Sidman RL (1972) New mutant mouse with communicating hydrocephalus and secondary aqueduct stenosis. Acta Neuropathol (Berl) 21:316–331

Bryan JHD, Hughes RL, Bates TJ (1977) Brain development in hydrocephalic-polydactyl, a recessive pleiotropic mutant in the mouse. Virchows Arch [A] 374:205–214

Grüneberg H (1943) Two new mutant genes in the house mouse. J Genet 45:22–28

Grüneberg H, Wickramaratne GA (1974) A re-examination of two skeletal mutants of the mouse, vestigial-tail (vt) and

congenital hydrocephalus (ch). J Embryol Exp Morphol 31:207–222

Hochwald GM (1985) Animal models of hydrocephalus: recent developments. Proc Soc Exp Biol Med 178:1–11

Jones HC (1984) The development of hydrocephalus in the mouse. Z Kinderchir [Suppl 2] 39:87–88

Jones HC (1985) Cerebrospinal fluid pressure and resistance to absorption during development in normal and hydrocephalic mutant mice. Exp Neurol 90:162–172

Jones HC, Cawkwell RD, Ellis C (1986) A modification of ependyma: interependymal pores in the roof of the fourth ventricle during mammalian development. Wiss Z Karl-Marx Univ Leipz Math Naturwiss Reihe (in press)

Kesterson JW, Carlton WW (1970) Aqueductal stenosis as the cause of hydrocephalus in mice fed the substituted hydrazine, cuprizone. Exp Mol Pathol 13:281–294

McClone DG, Bondareff W (1975) Developmental morphology of the subarachnoid space and contiguous structures in the mouse. Am J Anat 142:273–294

Nielsen SL, Baringer JR (1972) Reovirus-induced aqueductal stenosis in hamsters. Lab Invest 27:531–537

Punt J, Pickard J, Weller R, Mitchell JM, Mather C, Hunt R (1982) The hydrocephalic mutant mouse. J Neurol Neurosurg Psychiatry 45:280

Raimondi AJ, Bailey OT, McClone DG, Lawson RF, Echeverry A (1973) The pathophysiology and morphology of murine hydrocephalus in hy-3 and ch mutants. Surg Neurol 1:50–55

Raimondi AJ, Clarke SJ, McClone DG (1976) Pathogenesis of aqueductal occlusion in congenital murine hydrocephalus. J Neurosurg 45:66–77

Russell DS (1949) Observations on the pathology of hydrocephalus. Medical Research Council SR5 265, H.M.S.O., London

White DN, Wilson KC, Curry GR, Stevenson RJ (1979) The limitation of pulsatile flow through the aqueduct of Sylvius as a cause of hydrocephalus. J Neurol Sci 42:11–51

Williams B (1982) Aqueduct stenosis. Recent Adv Neuropathol 2:273–296

Received March 18, 1986/Accepted August 18, 1986