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# THE ONTOGENETIC DEVELOPMENT OF CONCENTRATION DIFFERENCES FOR PROTEIN AND IONS BETWEEN PLASMA AND CEREBROSPINAL FLUID IN RABBITS AND RATS

BY O. AMTORP AND S. C. SØRENSEN

*From the Institute of Medical Physiology, Department A University of Copenhagen, Copenhagen, Denmark*

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SUMMARY

1. The purpose of this study was to study in rats and rabbits the ontogenetic development of the blood–brain barrier to macromolecules and the ontogenetic development of concentration differences between plasma and cerebrospinal fluid for ions which are known to be transported actively across the choroid plexus and the blood–brain barrier.

2. By comparing the development of concentration differences for ions with the development of the blood–brain barrier to macromolecules we wanted to evaluate an eventual relationship between the development of these two functions of the blood–brain barrier.

3. The concentration of protein in cerebrospinal fluid and plasma was measured in foetal, juvenile and adult rabbits and in new-born, juvenile and adult rats. The concentration of protein was similar in rabbit foetuses at 23 days of gestational age (term at 31 days) and in new-born rats, and the ratio decreases at approximately the same rate in the two species.

4. The high concentration of proteins in cerebrospinal fluid might reflect either a high rate of entry of protein into the brain or a low production rate of cerebrospinal fluid. Injection of Diamox® (100 mg/kg) 2 hr before sampling of cerebrospinal fluid did not change the concentration of protein in cerebrospinal fluid in new-born rats whereas it increased the concentration in older rats. This finding suggests that new-born rat produces little (if any) cerebrospinal fluid suggesting that the high concentration of protein in cerebrospinal fluid in new-born rats reflect a low rate of turnover of cerebrospinal fluid.

5. The concentration of sodium, potassium, chloride and magnesium in plasma and cisternal cerebrospinal fluid was measured in rabbits of

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different age, from 23 days of gestation until adulthood, and in rats of different ages from birth until adulthood.

6. Concentration differences between plasma and cerebrospinal fluid for these were established in the youngest animals examined, indicating that the active transport mechanisms for these ions were functioning at an age where the concentration of protein in cerebrospinal fluid was very high.

7. The maintenance of concentration differences for ions at a time where the concentration of proteins in cerebrospinal fluid is high, is difficult to explain if the high concentration of proteins in cerebrospinal fluid is due to a leakiness of the intercellular junctions between cerebral endothelial cells. However, the findings might be explained either by a low rate of production of cerebrospinal fluid in the youngest animals and/or by a pinocytotic transfer of proteins across the blood–brain barrier in these animals.

8. In rats concentration gradients for ions are established at an age (new-borns) with a low or absent bulk formation of cerebrospinal fluid and at an age where the capillaries are still not enveloped by astrocytic foot processes. These facts suggest that the active transport mechanisms for the ions must be located in the cerebral endothelial cells.

## INTRODUCTION

The concept of a blood–brain barrier arose from the demonstration that protein-bound dyes do not penetrate from blood into the brain except in certain circumscribed areas, e.g. area postrema and median eminence. The morphological basis for this barrier to protein is the tight junctions between cerebral endothelial cells and between the 'epithelial' cells in the choroid plexus (Reese & Karnowsky, 1967; Brightman & Reese, 1969). Brain extracellular fluid, however, also differs from the plasma with regard to its ionic composition indicating the presence of active ion transport systems in the blood-brain barrier (Davson, 1970*b*; Bito & Myers, 1970; Katzman & Pappius, 1973; Davson & Hollingsworth, 1973).

The present study was undertaken in order to examine possible differences in the ontogenetic development of the barrier function for macromolecules and in the transport processes for ions between blood and cerebrospinal fluid in rabbits and rats. An attempt to evaluate the development of the barrier to macromolecules was made by determining the ratio between the concentrations of proteins in cerebrospinal fluid and plasma as a function of age. The development of transport processes for ions was assessed from the development of concentration differences for sodium, potassium, chloride and magnesium in cerebrospinal fluid and plasma.

The determination of the time course of development of the barrier function for macromolecules and of transport processes for ions responsible for the maintenance of concentration gradients between blood and brain extracellular fluid, would provide information which could be of help in locating the site of the transport systems for ions.

## METHODS

Foetal, juvenile and adult white rabbits and new-born, juvenile and adult Sprague–Dawley rats were used.

In rabbits the foetuses were removed from the mothers by Caesarean section at 23, 25 and 27 days of gestation (term at 31 days). The mothers were anaesthetized with pentobarbitone administered I.V. (30–60 mg/kg). Immediately after removing the foetus from the mother by Caesarean section cerebrospinal fluid was sampled from the cistern with a glass capillary. Thereafter a blood sample was obtained by heart puncture with a glass capillary or a syringe. Samples were taken only from foetuses with vigorously beating hearts.

Juvenile rats and rabbits were anaesthetized with pentobarbitone administered I.P. (40–80 mg/kg), and samples of cerebrospinal fluid and blood were obtained by cisternal puncture and heart puncture respectively.

Adult rats and rabbits were anaesthetized with pentobarbitone administered I.P. and I.V. respectively (30–60 mg/kg). Blood was sampled through a catheter in the carotid artery and cerebrospinal fluid was simultaneously sampled by cisternal puncture.

Samples of cerebrospinal fluid which were visibly contaminated with blood were discarded. This should ensure a blood concentration of less than 0·1 %.

*Measurement of protein concentration in cerebrospinal fluid and plasma.* The protein concentration in plasma and cerebrospinal fluid was determined by the method described by Lowry, Rosebrough, Farr & Randall (1951) as modified by Brenner, Falchuuk, Klimowitz & Berliner (1969). In rabbits the concentration of albumin and $\gamma$-globulin in plasma and cerebrospinal fluid was also determined. This was done by the immunochemical radial diffusion method (Mancini & Hermans, 1965). Antibodies against rabbit albumin were produced by immunization of goats. The purity of the antibody preparations were ascertained by immunoelectrophoresis. Antibodies against rabbit $\gamma$-albumin were obtained from DAKO, Copenhagen, Denmark. Rabbit albumin and rabbit $\gamma$-globulin obtained from Boehringerwerke, Germany were used for standards.

In a series of experiments Diamox® was injected I.P. (100 mg/kg) into rats of different ages 2 hr before sampling of blood and cerebrospinal fluid.

*Measurement of ion concentrations in cerebrospinal fluid and plasma.* The concentration of sodium and potassium ions in plasma and cerebrospinal fluid was measured with a flame photometer (I.L. Instruments) using the micropipetting system described by Siggård-Andersen & Brain (1967). 5 $\mu$l. of sample was used for each determination. The chloride concentration was measured by coulombmetric titration (Siggård-Andersen & Brain, 1967). 10 $\mu$l. of sample was used for each determination. The magnesium concentration was measured by atomic absorption flame photometry (Perkin Elmer 303). 25 $\mu$l. of sample was used for each determination. Thus for this analysis it was necessary to pool samples from the smaller animals in order to obtain sufficient amounts for duplicate determinations. All analysis were performed in duplicate.

The water content of foetal, new-born, juvenile and adult plasma was determined by drying the plasma at 105° C for 24 hr and the concentration of the ions in plasma water was calculated from the measured plasma concentration.

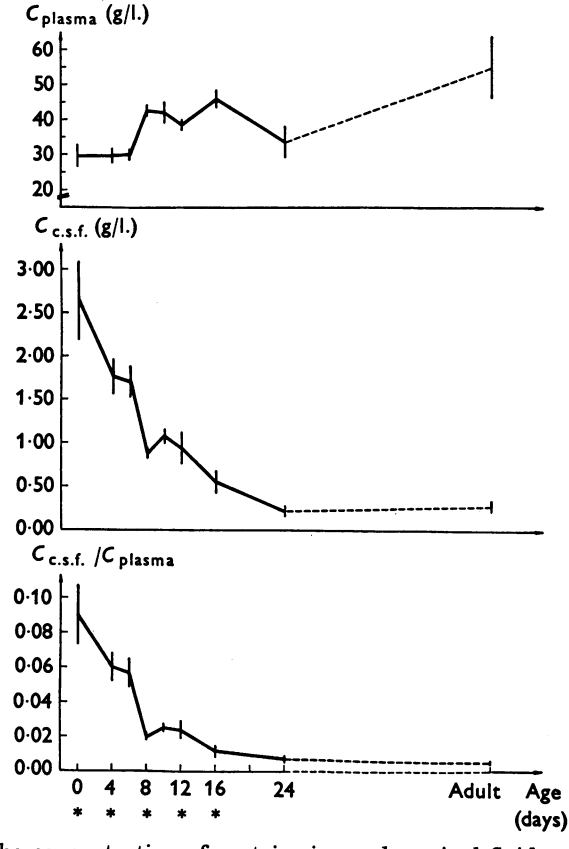


Fig. 1. The concentration of proteins in cerebrospinal fluid and plasma in rats at different ages. The bars indicate 1 S.D. The asterisks indicate that the values are significantly different from those in adult rats.

## RESULTS

The concentration of proteins in cerebrospinal fluid and plasma and the ratios between these concentrations in new-born, juvenile and adult rats and in foetal, juvenile and adult rabbits are shown in Figs. 1, 2 and 3. The ratio in rabbit foetuses at 23 days of gestational age is similar to that in new-born rats and the ratio decreases with age at approximately the same rate in the two species. In the rabbit the ratio is significantly higher than the adult ratio until 12 days of age ($P < 0{\cdot}05$). In rats the ratio is significantly higher than the adult value until the rats are 24 days old ($P < 0{\cdot}01$).

The effect of administration of acetazolamide (Diamox) on the concentration of proteins in cerebrospinal fluid was examined in rats in an attempt to evaluate possible changes in the rate of production of cerebrospinal fluid during development. The results of these experiments are shown in Fig. 4. They demonstrate that administration of acetazolamide (100 mg/kg) does not change the protein concentration in cerebrospinal

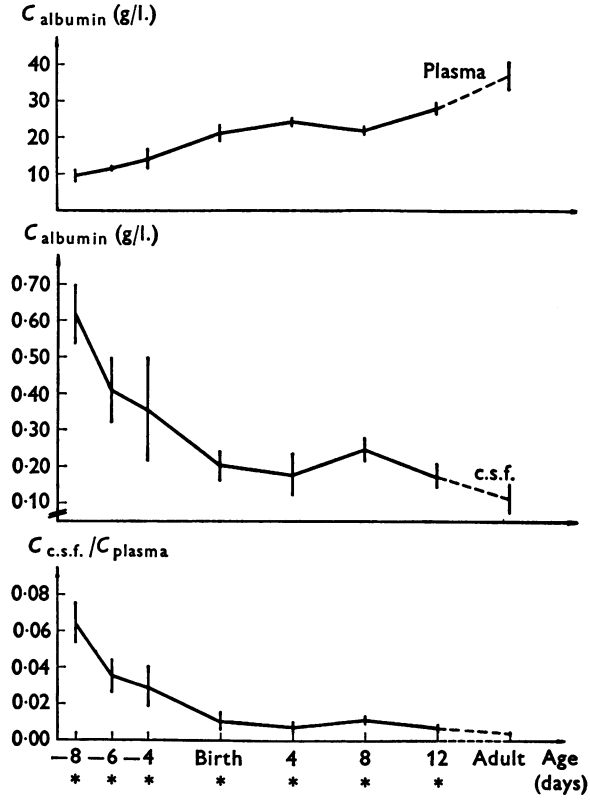


Fig. 2. The concentration of albumin in cerebrospinal fluid and plasma in rabbits at different pre- and post-natal ages. The bars indicate ± 1 S.D. The asterisks indicate values significantly different from adult values.

fluid in new-born rats but that it increases the concentration of protein in cerebrospinal fluid in the mature rats and that the relative increase in protein concentration is related to the age of the animals.

The concentration of sodium, potassium, chloride and magnesium ions in cerebrospinal fluid and plasma and the ratios between these concentrations in new-born, juvenile and adult rats and in foetal, juvenile and adult rabbits are shown in Figs. 5–11. The general pattern which is apparent

from the results shown in the Figures is that concentration differences for the different ions between plasma water and cerebrospinal fluid are established in the youngest animals which we have studied. The concentration ratios show variations during development, but that does not alter the general conclusion. It should be noted that the concentration ratio for potassium is lower in foetal and young rabbits than in adult rabbits.

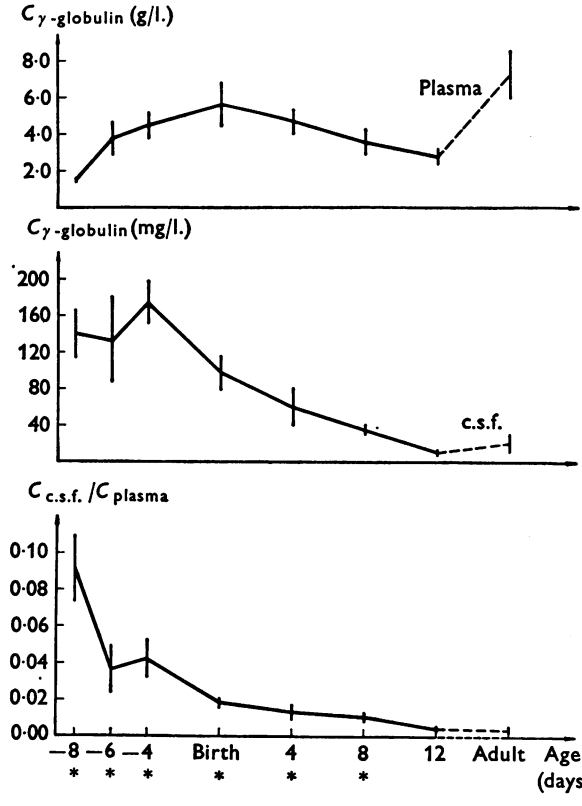


Fig. 3. The concentration of $\gamma$-globulin in cerebrospinal fluid and plasma in rabbits at different pre- and post-natal ages. The bars indicate 1 S.D. The asterisks indicate values significantly different from adult values.

The changes in the concentration ratio, however, mainly reflect changes in the plasma concentration since the concentration of potassium in cerebrospinal fluid remains stable throughout development. In rats the variations in concentration ratio for potassium also reflect variations in the plasma concentration because the concentration of potassium in cerebrospinal fluid remains stable from the time of birth until adulthood.

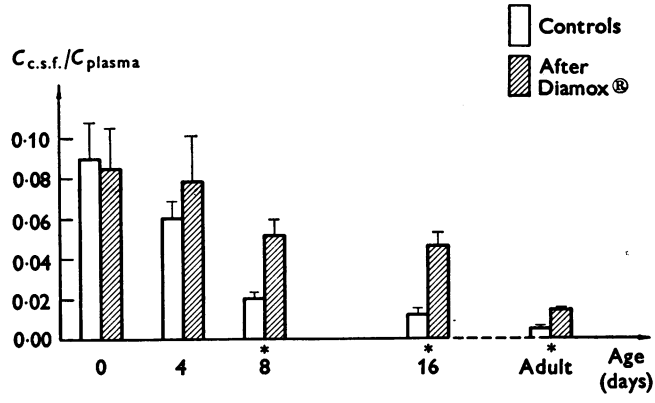


Fig. 4. The effect of Diamox on the protein concentration in cerebrospinal fluid in rats at different ages. The bars indicate ± 1 S.D.

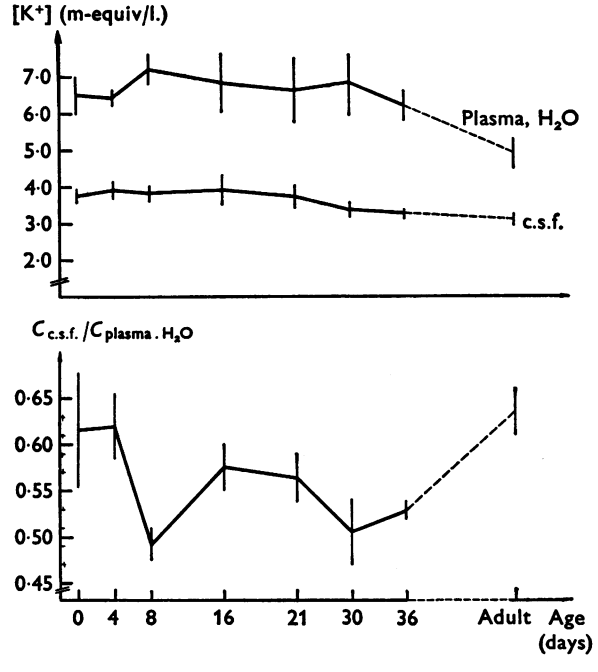


Fig. 5. Concentrations of potassium in plasma and cerebrospinal fluid from rats at different ages. The bars indicate ± 1 S.D.

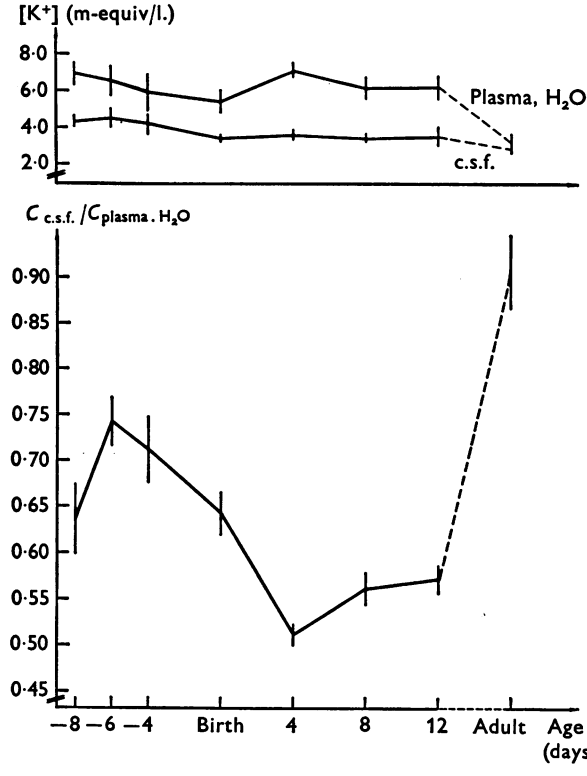


Fig. 6. Concentrations of potassium in plasma and cerebrospinal fluid from rabbits at different ages. The bars indicate ± 1 S.D.

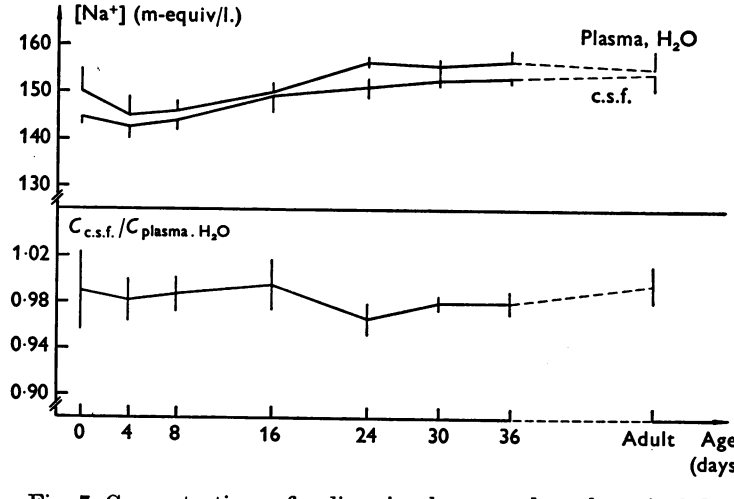


Fig. 7. Concentrations of sodium in plasma and cerebrospinal fluid from rats at different ages. The bars indicate ± 1 S.D.

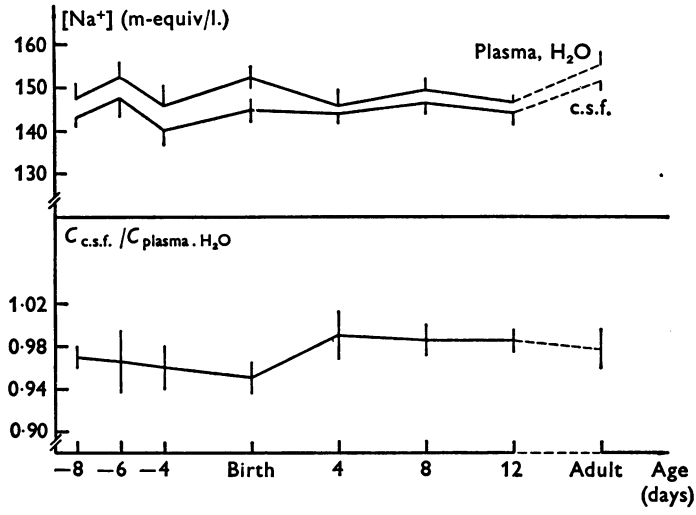


Fig. 8. Concentrations of sodium in plasma and cerebrospinal fluid from rabbits at different ages. The bars indicate ± 1 S.D.

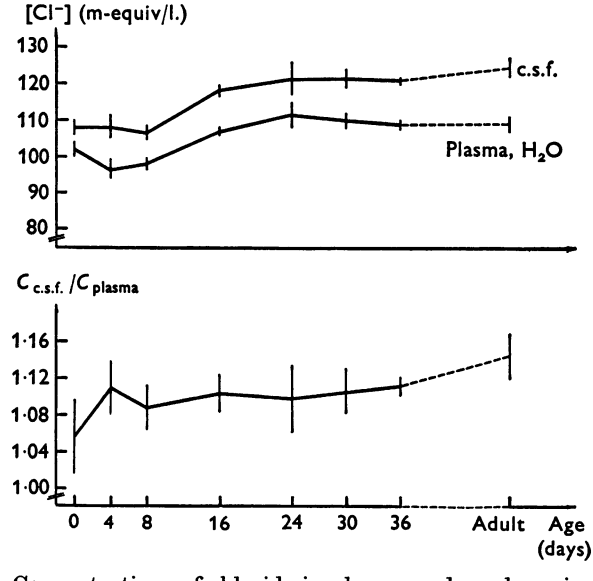


Fig. 9. Concentrations of chloride in plasma and cerebrospinal fluid from rats at different ages. The bars indicate ± 1S.D.

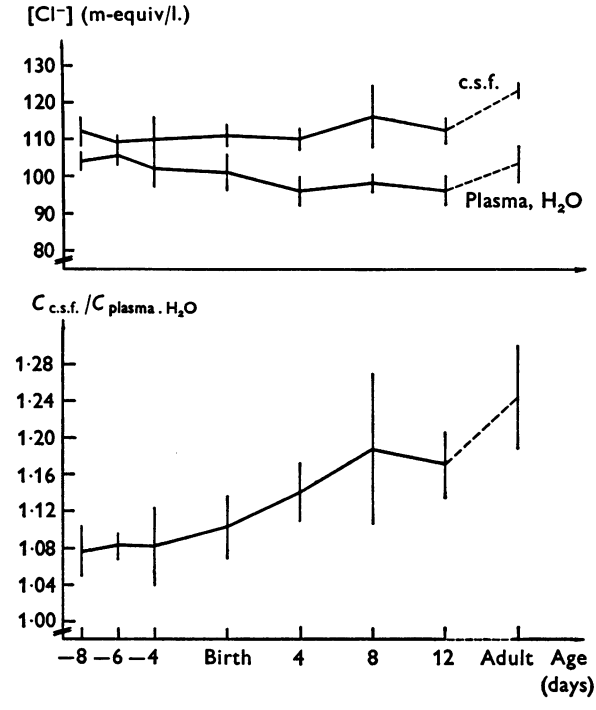


Fig. 10. Concentrations of chloride in plasma and cerebrospinal fluid from rabbits at different ages. The bars indicate ± 1 S.D.

## DISCUSSION

This study was an attempt to compare the ontogenetic development of the blood–brain barrier to proteins in rats and rabbits with the development of the ability to maintain concentration differences for ions between plasma and cerebrospinal fluid. We have studied rats and rabbits because they differ in terms of maturity at the time of birth.

It is known that the concentration of proteins in cerebrospinal fluid is higher in new-born children than in adults (Davson, 1970*a*) and it has been suggested that this is the result of a high permeability of the blood-brain barrier to proteins at this stage of development. The present study has shown that foetal rabbits and new-born rats also have a high concentration of proteins in cerebrospinal fluid. The ratio between the concentrations in cerebrospinal fluid and plasma is similar in rabbits at 23 days of gestational age and in new-born rats and this ratio decreases at approximately the same rate in the two species.

The concentration of proteins in cerebrospinal fluid is, however, determined both by the rate of entry of proteins from plasma into brain extra-

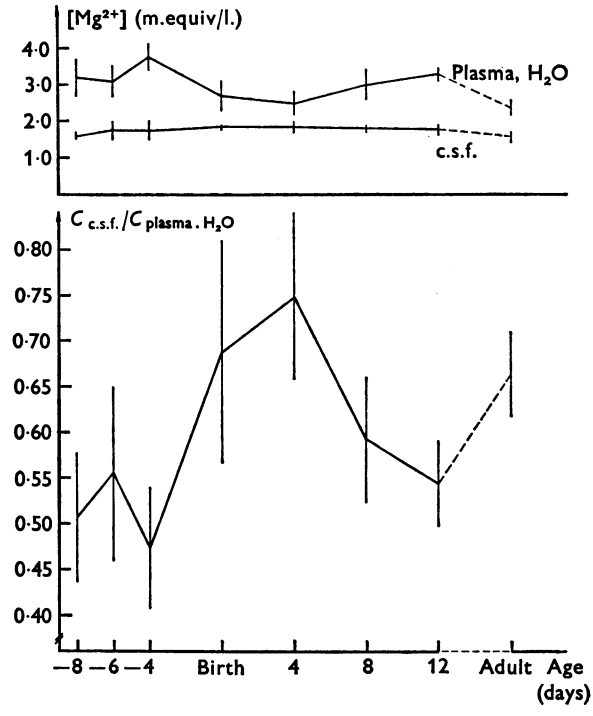


Fig. 11. Concentrations of magnesium in plasma and cerebrospinal fluid from rabbits at different ages. The bars indicate ± 1 S.D.

cellular fluid and by the rate of elimination from brain extracellular fluid. The rate of entry is determined by the 'permeability' of the blood–brain barrier and the rate of elimination is determined by the rate of bulk reabsorption of cerebrospinal fluid (i.e. cerebrospinal fluid production rate). Thus either of these two possibilities must be considered in evaluating the cause of the high protein concentration in cerebrospinal fluid in the young animals.

If the high protein concentration is due to a high 'permeability' of the blood–brain barrier, this might either be due to a greater leakiness of the intercellular junctions between cerebral endothelial cells or it might be caused by a greater pinocytotic activity in cerebral vessels in young animals. There is no evidence for a greater leakiness of the intercellular junctions in young animals and to the contrary Carley & Maxwell (1970) have found that the endothelial cells in the primitive capillaries in rat cerebral cortex are connected with tight junctions. We have not been able to find any information about the morphology of the capillaries in foetal rabbits.

Pinocytosis is probably of little importance for the transport of macromolecules between blood and brain extracellular fluid in adult animals because cerebral capillaries are characterized by a low number of vesicles in the endothelial cells (Brightman & Reese, 1969) in contrast to for instance muscle capillaries (Simionescu, Simionescu & Palade, 1974). However, portions of cerebral arterioles in the adult mouse brain are able to transfer large protein molecules such as horseradish peroxidase and ferritin from blood to brain extracellular fluid by vesicular transport (Westergaard & Brightman, 1973). It has also been shown that increased permeability of the blood–brain barrier to macromolecules following poisoning with either nickel chloride or mercuric chloride is accompanied by an increased number of coated vesicles in the cerebral capillaries (Joò, 1971). Thus it is possible that the high protein concentration in cerebrospinal fluid in immature animals reflects a greater transcapillary pinocytotic transport of macromolecules in immature animals compared with mature animals.

The rate of elimination of proteins from brain extracellular fluid is determined by the rate of production of cerebrospinal fluid. The present results suggest that the high concentration of protein in cerebrospinal fluid in new-born rats can be related to a decreased rate of production of cerebrospinal fluid. The absence of an effect of Diamox® on the protein concentration in cerebrospinal fluid in new-born rats suggest that they produce little if any cerebrospinal fluid. This conclusion is supported by the finding that the rate of turnover of cerebrospinal fluid in 5 days old rats is only 40% of that found in 30 days old rats (Bass & Lundborg, 1973). Thus at least in the rat the results might be explained by a low rate of turnover of cerebrospinal fluid. No information is available about the rate of turnover in foetal rabbits.

In the new-born rat the blood–brain barrier is apparently fairly tight to proteins (Olsson, Klatzo, Sourander & Steinwall, 1968) and the ability of the new-born rat to maintain concentration differences for the different ions might therefore not be so surprising. The finding may, however, be viewed in relation to the morphology of the new-born rat brain. In the new-born rat the endothelial cells in the primitive 'capillaries' are connected by tight junctions but the 'capillaries' are not surrounded by astrocytic foot processes as in the mature brain (Donahue & Pappas, 1961; Carley & Maxwell, 1970). Thus the fact that the new-born rats are able to maintain concentration differences for ions suggest that the transport mechanisms responsible for maintenance of these concentration differences are located in the endothelial cells. Therefore the cerebral endothelial cells have 'epithelial' functions and the finding rebuts the suggestion of Pappenheimer (1970) that the active transport mechanisms for ions are located in the perivascular astrocytes.

As mentioned previously we have not been able to find any information about the fine structure of the capillaries in foetal rabbits; thus we cannot explain a possible high rate of entry of proteins into the foetal rabbit brain on morphological grounds. However, it seems inconceivable that the rabbit foetuses should be able to maintain concentration differences for ions if the intercellular junctions between cerebral endothelial cells were highly permeable to proteins. A high rate of entry of proteins from plasma into the brain in foetal rabbits could be the result of a high pinocytotic activity in the cerebral vessels, but no evidence is available and more direct studies, morphological and physiological, are required to clarify this suggestion.

The concentration differences for different ions between plasma and cerebrospinal fluid during development have previously been studied in rats, monkey and sheep (Ferguson & Woodbury, 1969; Bito & Myers, 1970; Bradbury, Crowder, Desai, Reynolds, Reynolds & Saunders, 1972). Ferguson & Woodbury (1969) found in rats that the adult value for the concentration ratio for potassium was established 3 days after birth. In the rhesus monkey Bito & Myers (1970) found a temporal dissociation of the development of concentration differences for potassium, calcium and magnesium, but the concentration ratios for all three ions had reached the adult value at birth. In the sheep the concentration ratios for potassium, calcium and magnesium had reached the adult value in the middle of the second trimester.

All these results indicate that the transport systems which are responsible for maintaining concentration differences for ions across the blood–brain–c.s.f. barrier are functioning at an early stage of development.

## REFERENCES


Bass, N. H. & Lundborg, P. (1973). Postnatal development of bulk flow in the cerebrospinal fluid system of the albino rat: Clearance of carboxyl-($^{14}$C) inulin after intrathecal infusion. *Brain Res.* **52**, 323–332.

Bito, L. Z. & Myers, R. E. (1970). The ontogenesis of haematoencephalic cation transport processes in the rhesus monkey. *J. Physiol.* **208**, 153–170.

Bradbury, M. W. B., Crowder, J., Desai, S., Reynolds, J. M., Reynolds, M. & Saunders, N. R. (1972). Electrolytes and water in the brain and cerebrospinal fluid of foetal sheep and guinea-pig. *J. Physiol.* **227**, 591–610.

Brenner, B. M., Falchuuk, K. H., Klimowitz, R. I. & Berliner, R. W. (1969). The relationship between peritubular capillary protein concentration and fluid reabsorption by renal proximal tubule. *J. clin. Invest.* **48**, 1519–1531.

Brightman, H. W. & Reese, T. S. (1969). Junctions between intimately apposed cell membranes in the vertebrate brain. *J. cell Biol.* **40**, 648–677.

Carley, W. D. & Maxwell, D. S. (1970). Development of the blood vessels and extracellular spaces during postnatal maturation of rat cerebral cortex. *J. comp. Neurol.* **138**, 31–48.

DAVSON, H. (1970*a*). The normal protein concentration. In *Physiology of the Cerebrospinal Fluid*, pp. 272–273. London: Little, Brown and Company.

DAVSON, H. (1970*b*). Chemical composition and secretory nature of the fluid. In *Physiology of the Cerebrospinal Fluid*, chap. 2, pp. 33–54. London: Little, Brown and Company.

DAVSON, H. & HOLLINGSWORTH, J. R. (1973). Active transport of $^{131}I$ across the blood–brain barrier. *J. Physiol.* **233**, 327–347.

DONAHUE, S. & PAPPAS, G. D. (1961). The fine structure of capillaries in the cerebral cortex of the rat at various stages of development. *Am. J. Anat.* **108**, 331–347.

FERGUSON, R. K. & WOODBURY, D. M. (1969). Penetration of $^{14}C$ inulin and $^{14}C$ sucrose into brain, cerebrospinal fluid and skeletal muscle in developing rats. *Expl Brain Res.* **7**, 181–194.

JOÒ, F. (1971). Increased production of coated vesicles in the brain capillaries during enhanced permeability of the blood–brain barrier. *Br. J. exp. Path.* **52**, 646–649.

KATZMAN, R. & PAPPIUS, H. M. (1973). Cerebrospinal fluid ions. In *Brain Electrolytes and Fluid Metabolism*, chap. 5, pp. 75–110. Baltimore: The William and Wilkens Company.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with a folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

MANCINI, G. C. & HERMANS, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* **2**, 235–240.

OLSSON, Y., KLATZO, I., SOURANDER, P. & STEINWALL, O. (1968). Blood–brain barrier to albumin in embryonic, newborn and adult rats. *Acta neuropath.* **10**, 117–122.

PAPPENHEIMER, J. R. (1970). Transport of $HCO_3^-$ between brain and blood. In *Capillary Permeability, Alfred Benzon Symposium II*, ed. CRONE, C. & LASSEN, N. A., pp. 454–458. Copenhagen: Munksgaard.

REESE, T. S. & KARNOWSKY, M. J. (1967). Fine structural localization of a blood-brain barrier to exogenous peroxydase. *J. cell Biol.* **34**, 207–217.

SIGGÅRD-ANDERSEN, O. & BRAIN, S. B. (1967). Semiautomatic pipetting of ultramicro volumes of samples and reagent. *Am. J. clin. Path.* **48**, 502–509.

SIMIONESCU, M., SIMIONESCU, N. & PALADE, G. E. (1974). Morphometric data on the endothelium of blood capillaries. *J. cell Biol.* **60**, 128–152.

WESTERGAARD, E. & BRIGHTMAN, M. W. (1973). Transport of protein across normal cerebral arterioles. *J. comp. Neurol.* **152**, 17–44.