J. Physiol. (1984), **353**, *pp.* 405–417
With 6 text-figures
Printed in Great Britain

ABSORPTION OF THE CEREBROSPINAL FLUID AND INTRACRANIAL COMPLIANCE IN AN AMPHIBIAN, *RANA PIPIENS*

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(*Received 11 January 1984*)

SUMMARY

1. Experiments have been carried out to investigate the absorption of the cerebrospinal fluid (c.s.f.) and the intracranial compliance in an amphibian, *Rana pipiens*, using infusions into the c.s.f. system through glass micropipettes.

2. Resistance to absorption of the c.s.f. was estimated by the constant rate infusion technique. Mean absorption resistance for infusions of artificial c.s.f. into the lateral ventricles and into the cerebral subarachnoid space were 15·48 and 16·52 mmH_2O min μl^{-1} respectively. This difference was not significant and it is concluded that the pores in the posterior tela situated in the roof of the fourth ventricle do not offer any resistance to the flow of c.s.f. out of the ventricles. The resistance to drainage of the c.s.f. in this amphibian is higher than that found for mammals. Mean resting c.s.f. pressure, estimated from the intercept of the regression line with the pressure axis at zero infusion rate was 18·0 mmH_2O.

3. Absorption resistance was measured from the cerebral subarachnoid space before and after injection of 4 μl Indian ink solution. There was a 3-fold increase in resistance following ink injection. Two-way analysis of variance showed the difference to be significant ($P < 0{\cdot}01$) suggesting that the outflow sites can become partially blocked by particulate matter.

4. During a continuous 3 h infusion of artificial c.s.f. containing [^{14}C]dextran or [^{125}I]-labelled human serum albumin (RISA) into the lateral ventricles, the mean percentage uptakes into the systemic circulation after the first 0·5 h of a 3 h period were 74·1 and 61·9 % respectively. The difference is not significant. The rapid and high uptake into blood suggests there is a direct communication between c.s.f. and blood in amphibians.

5. During continuous infusion of RISA into the lateral ventricles, simultaneous blood samples were taken from the femoral artery and the internal dorsal vertebral vein. Radioactivity was found to be 13·2 % higher in venous samples. This suggests that at least some c.s.f. drainage takes place directly into the spinal venous system.

6. Intracranial compliance was investigated by recording the peak pressure in response to a series of bolus injections of artificial c.s.f. into one lateral ventricle. Compliance was estimated to be 0·11, 0·10 and 0·09 μl mmH_2O^{-1} for injection rates of 12·74, 16·62 and 25·10 μl min^{-1} respectively. The difference between these values

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is not significant. The results suggest that for injection volumes over 5 μl the c.s.f. system behaves elastically.

INTRODUCTION

The amphibian cerebrospinal fluid (c.s.f.) system is morphologically similar to that of mammals in many respects, consisting of both ventricular and subarachnoid c.s.f. (Cohen, Gerschenfeld & Kuffler, 1968; Cserr & Ostrach, 1974) the former of which is secreted by the choroid plexuses. In amphibians, however, the macroscopic foramina are absent, and the ventricular and subarachnoid fluids are connected by a system of interependymal pores in the caudal part of the membranous roof to the fourth ventricle, the posterior tela (Jones, 1978, 1979; Tornheim & Foltz, 1979). C.s.f. absorption sites analogous to the mammalian arachnoid villi have not been found in amphibians. In a study on the circulation of marker substances in the c.s.f. of *Rana pipiens* (Jones, 1980) it was shown that markers move rapidly out of the ventricular system into the subarachnoid space and that the region of the spinal nerve exits from the vertebral canal may be important for absorption of c.s.f. from the subarachnoid space. The study did not exclude the possibility that direct absorption into the spinal or cerebral veins was also taking place.

In mammals the resistance to absorption of the c.s.f. has been measured in a variety of species by both the constant rate and the constant pressure infusion methods (e.g. Davson, Hollingsworth & Segal, 1970). In the present study the constant rate infusion technique has been used in an amphibian to determine whether the c.s.f. has similar absorption characteristics to those of mammals and also to determine whether the pore-containing posterior tela provides a significant resistance to the flow of c.s.f. out of the ventricles. The uptake into blood of markers placed in the c.s.f. has also been investigated in order to study the site, rate and extent of transfer from c.s.f. to blood.

The distensibility or compliance of the brain and meninges has been measured in mammals by recording the rise in c.s.f. pressure in response to rapid changes in c.s.f. volume (e.g. Marmarou, Shulman & Rosende, 1978). The distensibility of the cranial compartment of *Rana pipiens* has been investigated in this study using a similar technique.

METHODS

Adult *Rana pipiens*, body weight 20–30 g, were anaesthetized in a water bath containing 1·5 mg ml^{-1} MS222 (tricaine methanesulphonate, Sandoz) buffered to pH 6–7. Animals were kept moist during experiments and additional anaesthetic was placed on the skin when required using cotton-wool swabs. All experiments were carried out at room temperature. The head was clamped and the body suspended at the pelvic girdle so that cranial and vertebral c.s.f. compartments were at the same level. A polyethylene cannula (Portex pp10, external diameter 0·61 mm) was inserted into the femoral artery and connected to a pressure transducer (Statham P37B) to record systemic arterial pressure. The skin was incised over the cranium and reflected. Holes were drilled in the skull above each cerebral hemisphere to expose the arachnoid and bevelled glass micropipettes (tip diameter 50–100 μm), clamped to micromanipulators, were lowered through the hemispheres into each lateral ventricle and held in place with cyanoacrylate adhesive. If sufficient care was taken during each insertion, the arachnoid formed a seal around the pipette and c.s.f. loss was avoided. One pipette was connected to a transducer (Statham P23BB) to record c.s.f. pressure and the other to a calibrated variable-speed pump (Braun Unita 1, Melsungen A.G., F.R.G.) fitted with a 500 μl gas-tight syringe (Hamilton Co.) for infusion. For some experiments, pipettes were placed in the

forebrain subarachnoid space instead of in the lateral ventricles. Frog artificial c.s.f. was used for infusions (in mM: NaCl, 83·6; KCl, 2·7; $CaCl_2$, 1·8; $MgSO_4$, 0·9; $NaHCO_3$, 25; at pH 7·4 and 200 mosmol) which, for some experiments, included one of the following marker substances: Evans Blue dye (2 mg ml^{-1}, E. Gurr), Blue Dextran (mol. wt. 2000000; 5 mg ml^{-1}, Pharmacia), carboxyl-[^{14}C]dextran (mol. wt. 70000; 10 μCi ml^{-1}, New England Nuclear) or [^{125}I]-labelled human serum albumin (RISA) (10 μCi ml^{-1}, Amersham).

Resistance to absorption

With micropipettes placed either in the lateral ventricles (twenty-four frogs) or in the forebrain subarachnoid space (eighteen frogs), constant rate infusions were made into the c.s.f. using six rates between 1·11 and 8·53 μl min^{-1}. During each infusion the c.s.f. pressure rose to a plateau level after which the pump was switched off and the pressure allowed to fall towards the resting level before restarting at a new rate (start–stop method). For each animal, the infusions were repeated using step-wise increases in rate, that is without stopping the pump between rates. It was established at an early stage that both start–stop and step-wise methods gave similar plateau pressures for a particular infusion rate. Both methods were used in each experiment, however, because a rapid fall in pressure after switching off the pump indicated patent, well positioned pipettes, whereas step-wise infusions were quicker to perform, and enabled a second set of measurements to be obtained for each animal. Pilot experiments had shown previously that varying the order of infusion rates had no effect on plateau pressure levels. The animals were grouped into three series according to the infusion solutions used: artificial c.s.f. alone, c.s.f. containing 2 mg ml^{-1} Evans Blue, or c.s.f. containing 5 mg ml^{-1} Blue Dextran. Each series was subdivided into animals for ventricular infusions and animals for subarachnoid space infusions. Absorption resistance was estimated for both start–stop and step-wise infusions from the gradient obtained by plotting plateau pressure against infusion rate by linear regression analysis and the mean of the two estimates was calculated for each animal.

In another series of six animals, a single pipette positioned in the forebrain subarachnoid space was connected to both the infusion pump and the pressure transducer with a T-piece adaptor. Prior to insertion, the pressure rise caused by the resistance to flow of the pipette plus tubing was measured at all infusion rates and the values obtained were subsequently subtracted from the experimental results. Step-wise infusions were carried out and c.s.f. absorption resistance was measured as described above. The pipette was then disconnected and approximately 4 μl Indian ink (particle diameter 0·2–1·0 μm) was back-filled into the tubing. The tubing was reconnected and the ink, followed by c.s.f., was infused into the subarachnoid space at 3·36 μl min^{-1} for 10 min. A second set of step-wise infusions were then performed and the absorption resistance recalculated. At the end of the experiments, four of these animals were perfused intravascularly with fixative (2% paraformaldehyde, 2·5% glutaraldehyde in 0·1 M-phosphate buffer), the heads were decalcified for 2 h in RDO (Du Page Kinetic Laboratories Inc.) and sectioned horizontally on a Vibrotome (Campden Instruments Ltd.). For the remaining two animals, the dura over the rostral end of the spinal cord was exposed prior to sacrifice by removing the atlas vertebra.

Treatment of radioactive samples

Whole blood samples (10 μl) taken from the femoral artery and samples of infusion solutions were weighed, digested overnight in 1 N-NaOH and the following day, neutralized with 1 N-HCl. For [^{14}C]dextran, samples were diluted to 0·5 ml with water and made up to 5·5 ml with Fisofluor-2 (Fisons Ltd.). For RISA, samples were diluted to 1·0 ml with water and made up to 11 ml with Fisofluor-2. The radiation was counted in a Packard Tricarb 460 scintillation counter with automatic quench correction. For blood samples, activity was calculated as disintegrations min^{-1} ml^{-1} plasma, using the haematocrit. This was measured at the beginning and end of experiments using 10 μl heparinized glass capillary tubes (Dade P4521-10) which were centrifuged at 3000 rev min^{-1} for 10 min.

Uptake from c.s.f. to blood

Preliminary experiments were carried out to measure the plasma volume and the extent to which radioactivity was lost from the blood compartment. For each of five animals, 0·5 μCi RISA was injected into the circulation through a polyethylene cannula inserted into the femoral vein. Blood samples were taken at timed 10–20 min intervals for 1·5 h from the femoral artery and a plot of

activity against time obtained. By extrapolation to time zero, the activity at the time of injection was calculated and used together with the haematocrit to estimate plasma volume. A mean value was obtained for plasma volume 100 g^{-1} body weight which was used to calculate plasma volume in frogs used for subsequent experiments. In three further experiments, a similar injection of [^{14}C]dextran or RISA was made and blood samples were taken at 20–30 min intervals for 3 h. This enabled the proportion of marker lost from the circulation during the 3 h period to be calculated.

For the uptake experiments, artificial c.s.f. containing [^{14}C]dextran (six frogs) or RISA (seven frogs) was infused into one lateral ventricle for up to 3 h at a constant rate (1·67 μl min^{-1}). This infusion resulted in a c.s.f. pressure rise which was normally very small and in no case greater than 30 mmH_2O. Blood samples (10 μl) were taken from the femoral artery at timed intervals of 20–30 min. For each sample the total activity in the blood was calculated from disintegrations min^{-1} ml^{-1} plasma × plasma volume and plotted against infusion time. The gradient of the straight line part of the plot was obtained by linear regression analysis and was equivalent to the rate of uptake of activity into blood in disintegrations min^{-1} min^{-1}. The percentage of marker transferred to blood for the duration of the experiment was obtained from the ratio of rate of uptake into blood to rate of entry into c.s.f. multiplied by 100.

In order to test for a direct route for absorption from c.s.f. to blood, a further four frogs were prepared before infusion by exposing the internal dorsal vertebral vein beneath the atlas vertebra and covering the surrounding tissues with a layer of cyanoacrylate adhesive to prevent any tissue fluid leakage. A continuous infusion of RISA into the lateral ventricles was carried out as described above. After the start of the infusion, blood samples were taken from the femoral artery every 20–30 min until around 80 min when a small incision was made in the internal dorsal vertebral vein and a blood sample was taken from this site simultaneously with one from the femoral artery. Plasma activity in the venous and arterial blood samples was measured and compared using two-way analysis of variance.

Compliance of the c.s.f. system

In seven frogs, pipettes were placed in both lateral ventricles. Bolus injections of frog artificial c.s.f. were made using three fast infusion rates (12·74, 16·62 or 25·10 μl min^{-1}) for a series of predetermined time intervals between 10 and 90 s. In this way the volume injected was varied between 2·77 and 37·65 μl. Between four and ten different volumes were injected at each infusion rate and the resulting c.s.f. pressure increases recorded. Each injection resulted in a rapid rise in c.s.f. pressure which showed little or no tendency to plateau. Following each injection, pressure was allowed to fall to resting level before a subsequent injection was made. For most experiments the maximum pressure was not allowed to exceed 200 mmH_2O. In each animal, compliance was calculated for each infusion rate as the reciprocal of the gradient obtained from a plot of pressure rise against volume injected using linear regression analysis.

RESULTS

Resistance to absorption

Each infusion rate caused an instantaneous rise in c.s.f. pressure which reached a steady state (or plateau level) within 3–10 min depending on pump speed. In all animals the relation between plateau pressure (20–200 mmH_2O) and infusion rate was found to be a linear one for both start–stop and for step-wise infusions (Fig. 1). The mean absorption resistances (Fig. 2) for artificial c.s.f. alone were $15{\cdot}48 \pm 2{\cdot}56$ ($n = 6$) and $16{\cdot}52 \pm 1{\cdot}46$ ($n = 6$) mmH_2O min μl^{-1} for ventricular and subarachnoid space infusions respectively. The corresponding figures for c.s.f. containing Evans Blue were $23{\cdot}54 \pm 2{\cdot}97$ ($n = 8$) and $20{\cdot}29 \pm 4{\cdot}80$ ($n = 6$) mmH_2O min μl^{-1} and for c.s.f. containing Blue Dextran were $21{\cdot}90 \pm 1{\cdot}88$ ($n = 10$) and $13{\cdot}7 \pm 1{\cdot}92$ ($n = 6$) mmH_2O min μl^{-1}. One-way analysis of variance showed that there was no significant difference between resistance measured from the ventricles and from the subarachnoid space for artificial c.s.f. alone or for c.s.f. containing Evans Blue. In the case of

Blue Dextran, however, ventricular infusions gave a significantly higher result ($P < 0{\cdot}01$). For Evans Blue infusions, the mean resistances for both ventricular and subarachnoid infusions were higher than for c.s.f. alone, as was that for ventricular infusions of Blue Dextran, but these differences were not statistically significant.

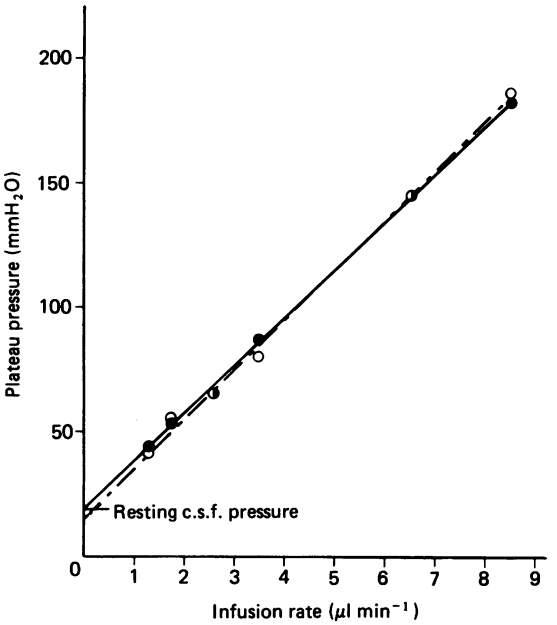

Fig. 1. A typical experiment to measure the resistance to absorption. Artificial c.s.f. was infused into the subarachnoid space using both the start–stop (●) and step-wise (○) methods and the plateau pressures plotted against infusion rate. Lines were drawn using linear regression analysis, and the resistances to absorption were calculated from the slopes.

By taking the value for the intercept of the regression line with the pressure axis (zero infusion rate) for each experiment (e.g. Fig. 1), the mean resting c.s.f. pressure for all animals was calculated to be $18{\cdot}0 \pm 5{\cdot}3$ mmH$_2$O ($n = 42$). This method was thought to be more reliable than a direct measurement of the pressure taken at the start of experiments because resting pressure may have been altered temporarily when the pipettes were inserted.

Resistance to absorption from the subarachnoid space before Indian ink injection was $13{\cdot}28 \pm 1{\cdot}56$ mmH$_2$O min μl^{-1} (Fig. 3). This rose to $40{\cdot}00 \pm 9{\cdot}90$ mmH$_2$O min μl^{-1} after ink injection and two-way analysis of variance showed the difference to be significant ($P < 0{\cdot}05$). Thick sections (150 μm) cut horizontally through fixed heads showed ink particles to be distributed throughout the c.s.f. system, including the ventricles and the subarachnoid space of both the brain and spinal cord. Accumulations of particles were noticeable in or around the cranial and spinal nerve roots but were not found outside the c.s.f. system. In the remaining two animals in which the dura was exposed there were ink particles in and around the dura. These appeared to have escaped from the spinal subarach-

noid space, although the arachnoid remained intact. In one of these experiments, arterial blood samples were taken at the end of the experiment and centrifuged in haematocrit tubes. No ink particles were visible.

Uptake from c.s.f. to blood

The mean plasma volume in five frogs was estimated to be $6{\cdot}403 \pm 0{\cdot}441$ ml 100 g^{-1} body weight; this value was used for the calculation of plasma volume for frogs used in uptake experiments. The proportion of injected radioactivity lost from the circulation over 3 h varied between 30 and 60%.

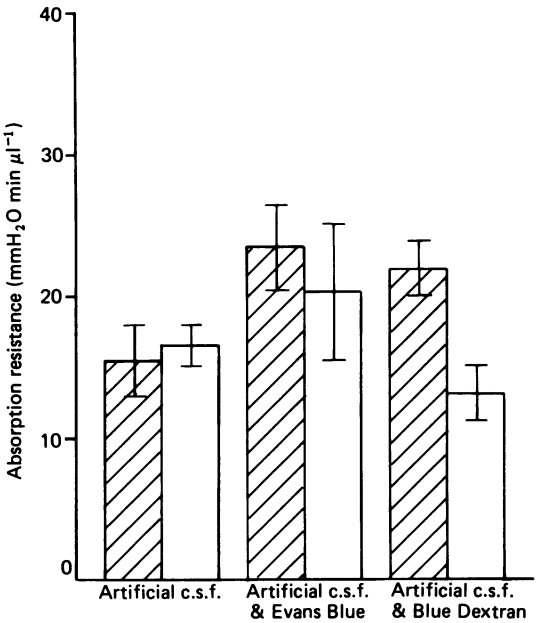

Fig. 2. Resistance to absorption for ventricular infusions (hatched bars) and subarachnoid space infusions (open bars) of three test solutions. Values are means ± S.E. of mean (n = 6–10).

For the uptake experiments, the first blood sample was taken approximately 30 min after the start of infusion into the c.s.f. A low level of activity was measured in the blood at this time which increased rapidly during the next 20 min (Fig. 4; dashed line). This initial 50 min period was assumed to be the time required for equilibration of the marker concentration in the c.s.f. system and corresponds to a period of rapidly increasing rate of entry into blood. After the first 50 min the rise in blood activity with time was linear (e.g. Fig. 4; continuous line) and percentage uptake rate was calculated using the gradient obtained from this part of the plot. For [^{14}C]dextran the uptake into blood after the initial equilibration period ranged from 51·7 to 92·4%, mean = $74{\cdot}1 \pm 6{\cdot}0$% (n = 6, Fig. 5). For RISA the range was 32·9–79·4%, mean = $61{\cdot}9 \pm 6{\cdot}3$% (n = 7). There was no significant difference between the percentage uptake rates calculated for the two radiolabelled markers.

For the experiments in which simultaneous blood samples were taken from the femoral artery and the internal dorsal vertebral vein during RISA infusion into the c.s.f., mean concentration for the venous samples expressed as a percentage of inflow concentration was $5{\cdot}2 \pm 0{\cdot}8$ % and for arterial samples was $4{\cdot}6 \pm 0{\cdot}7$ %. This difference is significant ($P < 0{\cdot}01$) and represents an activity which is higher in venous blood than in systemic arterial blood by 13·2 %.

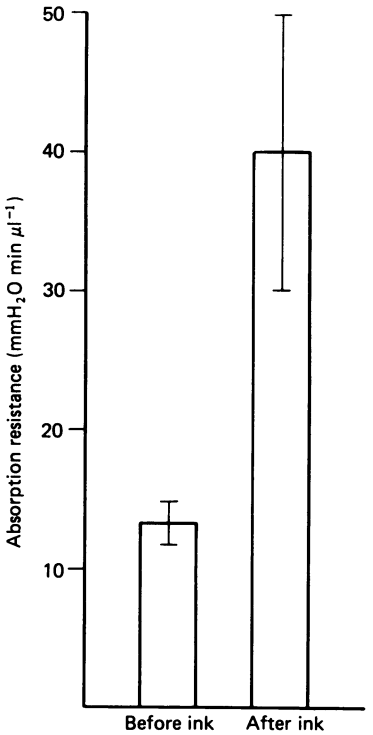

Fig. 3. Resistance to absorption measured using infusions of artificial c.s.f. into the subarachnoid space before and after injection of 4 μl Indian ink through the infusion cannula. Values are means $\pm$ S.E. of mean ($n = 6$).

Compliance of the c.s.f. system

The relationship between the rise in c.s.f. pressure in response to a bolus injection and the volume injected was, in most cases, a linear one for volumes between 5–10 and 20 μl (Fig. 6). For smaller volumes, compliance became greater as the graph was extrapolated back to zero. For volumes over 20 μl, which in some animals resulted in pressure increases greater than 200 mmH_2O, the pressure–volume relationship tended to become erratic, and in two experiments, a non-linear plot was obtained over the whole range of volumes used. In these cases compliance appeared to increase with the volume injected and this effect may have been due to an abnormal loss of c.s.f. through leakage or to a high rate of absorption. Compliance was calculated only from linear plots and, for individual animals there was good agreement between rates. Over-all mean values for compliance were $0{\cdot}11 \pm 0{\cdot}01$ ($n = 6$), $0{\cdot}10 \pm 0{\cdot}01$ ($n = 10$) and

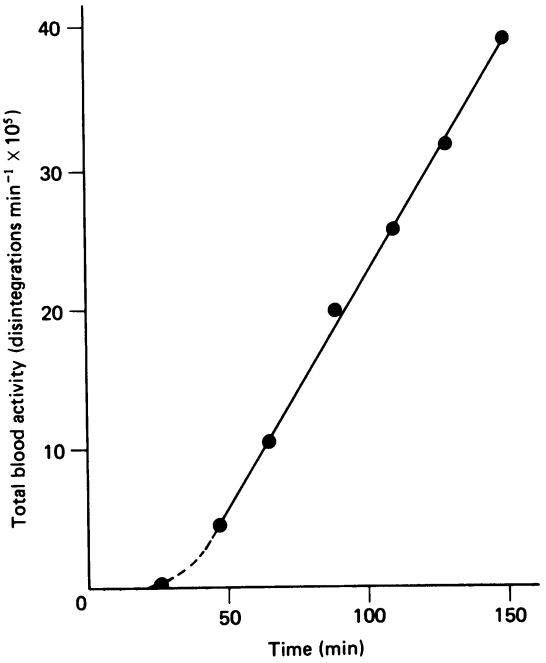

Fig. 4. A typical experiment showing the uptake of RISA into blood during infusion into the c.s.f. Total blood activity is plotted against infusion time and the rate of entry into blood was estimated as the gradient of the regression line for the linear part of the plot (50–150 min).

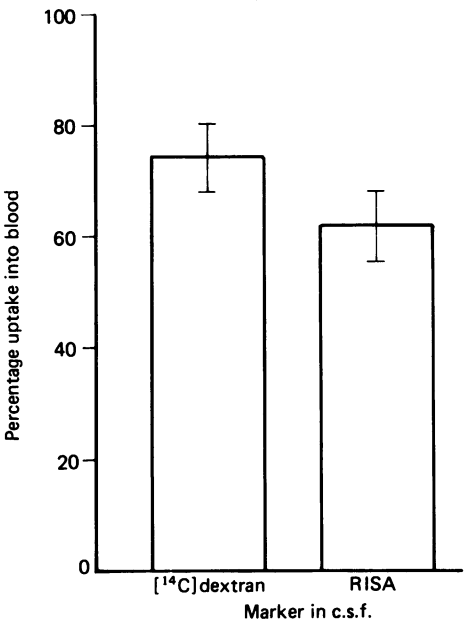

Fig. 5. Percentage uptake by blood for [^{14}C]dextran and for RISA infused at 1·67 μl min^{-1} into the lateral ventricle. Values are means ± S.E. of mean (n = 6 and 7).

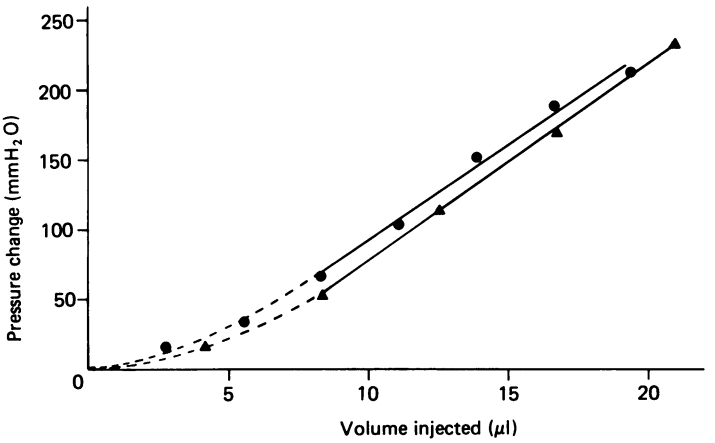

Fig. 6. Rise in c.s.f. pressure plotted against volume injected into the c.s.f. of one frog at two different rates. Compliance of the c.s.f. system was calculated as the inverse slope of the regression lines on the linear part of the plot (continuous lines). Extrapolation back to zero (dashed lines) represents higher compliance obtained with low volumes. ●, 16·62 μl min^{-1}; ▲, 25·10 μl min^{-1}.

0·09±0·01 (n = 9) μl mmH_2O^{-1} for injection rates of 12·74, 16·62 and 25·10 μl min^{-1} respectively. One-way analysis of variance showed no significant difference between compliance calculated for different injection rates.

DISCUSSION

Methods used in the past to measure the resistance to absorption of the c.s.f. include perfusion of the ventricular system at different pressures (Pappenheimer, Heisey, Jordan & Downer, 1962), bolus injections into the c.s.f. (Sullivan, Miller, Griffith, Carter & Rucker, 1979) and also infusion into the c.s.f. at either constant rate or constant pressure (Davson *et al.* 1970). Values obtained here for the frog using the constant rate infusion method (15–17 mmHg min μl^{-1}) fall close to those found for the turtle using the perfusion method (Heisey & Michael, 1971), but are lower than those obtained for the chicken (Anderson & Heisey, 1972) and slightly higher than those obtained for the rat (Mann, Cookson & Mann, 1980; Deane & Jones, 1983). Lower resistances have been found for all other mammals investigated, e.g. cat (Sullivan *et al.* 1979), dog (Mann, Butler, Rosenthal, Maffeo, Johnson & Bass, 1978), monkey (Blasberg, Johnson & Fenstermacher, 1981) and rabbit (Davson *et al.* 1970). Since c.s.f. absorption is probably related to the rate of fluid formation, then a high resistance could be anticipated for *Rana pipiens* where c.s.f. production rate is low at 0·2 μl min^{-1} (H. C. Jones & C. M. Taylor, unpublished observations).

In the present study a linear relationship was found between plateau pressure and infusion rate, that is the resistance to absorption was constant over the range of flow rates used. This contrasts with results obtained for the rat where resistance is pressure dependent and is reduced at high flow rates (Mann *et al.* 1978, 1980; Deane & Jones, 1983). The pressure-dependent outflow resistance in mammals has been

attributed to a valve-like phenomenon at the outflow sites. In support of this, Butler, Maffeo, Johnson & Bass (1981) have shown by electron microscopy that there is an increase in transendothelial vesicular transport across rat arachnoid villi in response to increases in c.s.f. pressure, which ultimately resulted in the formation of open channels. It seems possible that because resistance does not depend on pressure, the mechanism of outflow across amphibian drainage sites is different to that through mammalian arachnoid villi.

From studies on the morphology of the amphibian c.s.f. system (Jones, 1979) it was thought that the posterior tela, the membrane through which the c.s.f. flows from the ventricles into the subarachnoid space, might offer some resistance to flow. There was, however, no significant difference between resistance measured from the ventricles and from the subarachnoid space, when artificial c.s.f. was used as the infusion fluid. This shows that even at the higher flow rates the pores in the posterior tela ependyma are sufficiently large for unrestricted flow of c.s.f. Of the three solutions used in this study, the most consistent results were obtained with artificial c.s.f. alone. We have found recently that Evans Blue dye causes extreme morphological changes to the ependyma when infused into the ventricular system of *Rana pipiens* (Jones & Taylor, 1983). Thus it is possible that the higher resistance with c.s.f. containing Evans Blue may be due to dye-induced changes, either in the flow pathway leading to the outflow sites, or in the outflow sites themselves. Artificial c.s.f. containing Blue Dextran gave a significantly higher resistance from the ventricles than from the subarachnoid space, and because this difference was not found with c.s.f alone, it seems likely that it could be due to the high viscosity of the dextran solution. A similar effect with dextran has been seen during constant pressure infusions in the rabbit when the flow rate fell markedly on switching from c.s.f. to c.s.f. containing dextran (Davson *et al.* 1970).

In this study the infusion of Indian ink into the subarachnoid space resulted in a 3-fold increase in outflow resistance and there was no evidence that ink particles were entering the blood. This suggests that the outflow sites are such that they can become partially blocked by particulate matter 0·2–1·0 μm in diameter. Similar experiments performed in mammals have shown that small particles or red cells pass easily from c.s.f. to blood, for example, in rats 0·5 μm polystyrene beads had similar efflux characteristics to inulin (Mann, Butler, Johnson & Bass, 1979). In rabbits, labelled erythrocytes injected into the subarachnoid space passed directly into the circulation (Simmonds, 1953), and injection of Indian ink into the subarachnoid space of sheep resulted in particles passing through open channels to the venous side of arachnoid granulations (Jayatilaka, 1965). On the other hand, changes in resistance, similar in magnitude to those found here with ink particles, have been observed in mammals in response to infusions of whole blood or plasma (Davson *et al.* 1970; Butler *et al.* 1981; Blasberg *et al.* 1981). The effect was smaller or absent, however, if serum, plasma dialysate or heparinized blood was used, suggesting that the increase in resistance could, at least in part, be due to deposition of fibrin in the outflow sites.

The accumulation of ink particles around the spinal nerve roots is consistent with the results of a previous study using dextran (Jones, 1980). The presence of ink particles outside the arachnoid in and around the dura dorsal to the spinal cord, could have occurred either by traversing the membrane in a general way, or, as seems more

likely, by traversing the arachnoid at the site of transit by the spinal nerve roots (Jones, 1980).

After continuous infusion of radiolabelled marker into the lateral ventricle, radioactivity was detected in the blood around 30 min after the start of the infusion. When it is considered that time is required for the marker to reach the absorption sites and that it will be greatly diluted on entering the blood, this represents a rapid transfer from c.s.f. to blood and suggests that a direct communication exists between the two compartments. This is despite the fact that no arachnoid villi have hitherto been located in these animals. The variation in uptake percentage between animals was high; some of this could be accounted for by variations in plasma volume since the latter could not be measured for each experiment. Nevertheless the uptake percentages obtained for [^{14}C]dextran (mol. wt. 70000) and for RISA (mol. wt. 60000) were not significantly different which suggests that there is no discrimination at the outflow sites for these two chemically dissimilar molecules. If we take into account the exit of marker from the circulation of between 30 and 60 % for a similar time period a very high percentage of the infused activity is accounted for in the circulation. A few comparable studies have been carried out in mammals. Continuous infusion of RISA into the lateral ventricles of rabbits resulted in 37 % of the infused activity being recovered in the blood (McComb, Davson, Hyman & Weiss, 1982). After a single injection of RISA into c.s.f., the recovery in blood was found to be 35 % in rabbits and 42·7 % in cats (Bradbury & Cole, 1980). Other figures for recovery in blood of rabbits are 39 % over 6 h (Prockop, Shanker & Brodie, 1961) and 20 % over 3–5 h (Courtice & Simmonds, 1951). Thus the percentage recovery in blood for frogs is approximately twice that found for mammals.

In mammals, it has long been known that the subarachnoid space has connexions with the lymphatic system (Brierley & Field, 1948), and recently the drainage of c.s.f. into lymph has been estimated to be as high as 30 % in the rabbit (Bradbury & Cole, 1980; Bradbury & Westrop, 1983). A lymphatic route for c.s.f. drainage has been reported in the bull-frog (Tornheim & Foltz, 1979) but we have not been able to confirm this (Jones, 1980). We cannot, however, exclude the possibility that in these experiments, a proportion of marker did reach the blood via an indirect, lymphatic, route, particularly since lymph flow is important for amphibians and its turnover is rapid (Conklin, 1930).

We have found that during a continuous infusion into the lateral ventricles, blood samples taken from the internal dorsal vertebral vein had on average 13·2 % higher activity than simultaneous arterial samples. This suggests that there is direct passage of c.s.f. into the venous system. Similar results have been obtained by sampling superior sagittal sinus blood and systemic arterial blood in rats (Mann *et al.* 1979) and in cats (Courtice & Simmonds, 1951; Sahar, Hochwald & Ransohoff, 1970). It has been shown previously in amphibians that, from the ventricles, the bulk of the c.s.f. flows caudally into the spinal subarachnoid space and that markers tend to accumulate around the spinal nerve exit sites from the vertebral canal (Tornheim & Foltz, 1979; Jones, 1980). The internal dorsal vertebral vein at the sampling site used in this study, contains blood which drains from the spinal cord. Thus it seems likely that substantial drainage sites exist in the spinal cord subarachnoid space in frogs. Spinal arachnoid villi associated with spinal nerve sheaths have been found in

mammals (Welch & Pollay, 1961) and it is possible that analogous structures occur in amphibians.

The ability of the c.s.f. system to respond to rapid changes in volume or pressure depends on the distensibility, or compliance, of the various components of the system, that is the brain, meninges and skull. In order to measure compliance, the rate of injection of known volumes into the c.s.f. must be sufficiently high for the proportion of the injected volume which is lost through the absorption sites, to be insignificant. In this study the three injection rates gave similar values for compliance, indicating that these rates were fast enough for absorption to be negligible.

The relationship between volume injected and pressure rise consists of two components; a non-linear (high compliance) phase for volumes injected of less than 5 μl, and a linear (low compliance) phase for volumes between 5 and 20 μl. Thus for volumes below 5 μl the system is more distensible. This may be due to a limited volume accommodation by venous blood vessels within the brain or c.s.f. compartments. A similar two-phase system was found in the dog in which elastance (the reciprocal of compliance) became 20 times greater above a c.s.f. pressure of 15 mmHg (Lofgren, Essen & Zwetnow, 1973). More recently in mammals, using the same technique, an exponential pressure–volume curve has been found, the slope of which increases with volume (e.g. Marmarou *et al.* 1978; Sullivan *et al.* 1979). From this study, there is no evidence for an exponential relationship in the frog. In conclusion, intracranial compliance in *Rana pipiens* is constant when measured between volumes of 5–20 μl. This means the system behaves elastically. The skull is normally rigid in frogs but the subdural space which contains the endolymphatic sacs, is much larger than in mammals and might be compressed when the brain and meninges are distended thus giving the c.s.f. system a much greater elasticity than it would have otherwise.

The financial support of the SERC and the Wellcome Trust are gratefully acknowledged. We are grateful to Professor M. W. B. Bradbury for his comments on the manuscript.

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