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A FLOW OF CEREBROSPINAL FLUID ALONG THE CENTRAL CANAL OF THE SPINAL CORD OF THE RABBIT AND COMMUNICATIONS BETWEEN THIS CANAL AND THE SACRAL SUBARACHNOID SPACE

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The central canal of the spinal cord is generally regarded as a functionless remnant, containing stagnant cerebrospinal fluid (c.s.f.) and cellular debris. Indeed, it may become obliterated during the life of many species (Agduhr, 1932). Nevertheless, it has long been known that the ventricular ependyma is ciliated (Purkinje, 1836; Studnicka, 1900) and that in certain vertebrate species, if not all, cilia are also a feature of the ependyma of the central canal. A cilia-mediated circulation of c.s.f. within the ventricular cavity of *Xenopus* larvae has been reported (Adam, 1953), and movements of c.s.f. in the central canal suggested—the latter in conjunction with the caudally directed progress of Reissner's fibre along the central canal (Olsson, 1958).

Up till now, there has been no evidence to suggest that a flow of c.s.f. might occur in the central canal of any adult mammal. Observations made in this laboratory during the course of experiments on water and electrolyte exchange, employing the ventriculocisternal perfusion technique, which was developed in the goat by Pappenheimer, Heisey & Jordan (1961) and was applied to the rabbit by Pollay & Davson (1963), strongly suggested the possibility that, in the latter species at least, such a flow might occur. In order to outline the track of fluid flow during this procedure, Evans Blue was added to the inflowing perfusion fluid. When the central nervous system was exposed after 2 hr perfusion, not only was the ependyma of the cerebral ventricles and the pia mater adjacent to the cisterna magna stained blue, but dye had penetrated the length of the central canal of the spinal cord and had entered the filum terminale. This observation was interpreted as evidence of c.s.f. flow in the central canal, and the present experiments were undertaken in order to study the hypothesis further.

In these experiments the existence of a caudally directed flow of c.s.f. along the central canal has been demonstrated and the existence of communications between the canal and the sacral subarachnoid space shown

by means of injections of Evans' blue, ^{131}I-albumin and colloidal graphite into the lateral ventricle of the rabbit. Small-volume injections of Evans Blue into the ventricular system of a number of other adult mammals have failed to show any flow in the canal except in the rat in which animal there was penetration of a small amount of dye along its length.

The results of this work were demonstrated to the Physiological Society (Bradbury, Davson & Lathem, 1964).

METHODS

The majority of the experiments were carried out on adult albino rabbits, weighing between 2·0 and 3·5 kg. Anaesthesia was induced with intravenous sodium pentobarbitone (Nembutal, Abbott Laboratories) 30 mg/kg and open-drop ether. Anaesthesia was maintained with intravenous injections of Nembutal given as necessary. Three indicators were injected into the lateral ventricle—Evans Blue, ^{131}I-albumin and colloidal graphite. The technique was as follows:

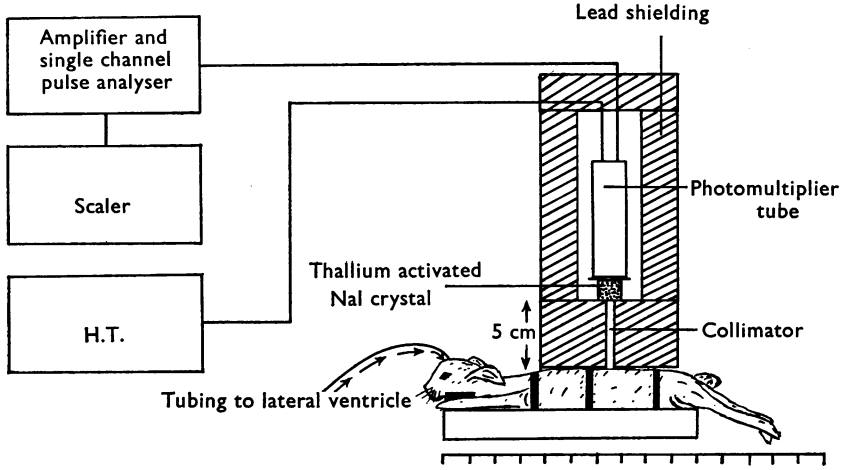
Evans Blue. The animal was placed on a cradle attached to a steel frame to which were fixed clamps for stabilizing the skull and vertebral column. The apparatus and technique for visualizing the Evans Blue at the end of the experiment without disturbing its distribution were essentially similar to those described by Grundy (1963). Clamps were attached from above to the head of the animal, the first three thoracic vertebral spines and the ilia just posterior to their crests. The nasion, opisthion and vertebral laminae of the animal were brought in line and the system made rigid. The vault of the skull was exposed and a fine drill hole made 10 mm behind the coronal suture and 9 mm from the mid line. A polythene catheter, which was a close fit in the hole (0·38 mm bore), was inserted through to a depth of approximately 7 mm. In seven experiments, 1 % Evans Blue in artificial c.s.f. (Bradbury & Davson, 1964) was injected through the catheter into the lateral ventricle at a rate of 0·067 ml./min with the constant speed injection machine of Davson & Purvis (1952). A low-pressure torsion wire manometer was incorporated in the system. If the pressure rose, the tip of the catheter was moved outwards a little; this was attended by a rapid drop in pressure which had been previously shown to be a reliable sign of correct placement of the catheter in the lateral ventricle (Pollay & Davson, 1963). Generally, a total of 0·2 ml. of 1 % Evans Blue was injected over 3 min. Once correct placement had been achieved, the injection was not associated with any detectable rise in ventricular pressure (manometer sensitive to changes of 2 cm water).

After 2 hr the animal was killed by an intravenous injection of 3 ml. of Nembutal. With the skull and vertebral column of the animal still clamped, the rest of the body was dissected away. The cradle was removed, and a bath containing freezing mixture (solid CO_2-ethanol) was brought up from underneath to immerse the specimen. Thirty minutes later the entire frozen specimen was sectioned transversely to its long axis at 2 cm intervals with a band-saw.

The distribution of the dye at the cut surfaces of the still-frozen segments could easily be seen and was noted. The segments were then placed in 10 % formalin for 24 hr, at the end of which time the cord, enclosed in dura mater, was dissected out. In the frozen specimens it was apparent that much of the dye was present in a layer of frozen c.s.f. between the C.N.S. and the dura mater. At dissection it appeared that ependyma, pia and arachnoid mater were stained in regions, where dye was present. Where much dye was present, the adjacent nervous tissue was stained to a depth of up to 1 mm. This seemed more likely to occur in pia-lined than in ependyma-lined areas. The distribution of the dye was assessed visually as marked, slight or nil and there was nearly complete agreement between assessments made from frozen and formalized specimens. The farthest penetration of dye in the central canal

and in both the dorsal and ventral subarachnoid space was related to the cord length, the posterior end of the IVth ventricle being taken as the anterior limit of the cord and the sacral cord at a distance of 1 mm as the posterior limit. In order to assess whether blue coloration in the lumbosacral space, noted during the intraventricular experiments, could be due to preferential staining of the tissues in this region by dye which had passed directly caudally in the spinal subarachnoid space, control experiments were performed. In these experiments, of which there were 3, a 14-gauge needle was inserted in the cisterna magna through the atlanto-occipital joint, the least quantity of c.s.f. required to establish correct entry was withdrawn with a suction device and 0·2 ml. of 1 % Evans Blue injected, as into the ventricle. Otherwise, the procedure was precisely similar to that of the intra-ventricular experiments.

^{131}I-labelled albumin. Rabbits were anaesthetized as above and laid prone on a wooden sledge which ran horizontally in grooves, parallel to the longitudinal axis of the animal. The head was slightly raised to the same position, as in the Evans Blue experiments, by means of a wooden head-rest screwed to the sledge. The animal was kept immobile by means of strips of adhesive plastic tape stretched across its back from one side of the sledge to the other. Vertically above the vertebral spines of the animal was fixed the collimator of a γ-scintillation counter. Thus, by sliding the sledge in its grooves it was possible to record γ-activity over any discrete part of the spinal cord.



Text-fig. 1. Apparatus for recording radioactivity over the spinal cord after the injection of ^{131}I-albumin (100 μc) into the lateral ventricle or the cisterna magna. The rabbit is strapped to a sledge which slides backwards and forwards in grooves.

The collimator was circular in cross-section and 1 cm in diameter. The lower surface of its shielding just touched the skin of the animal. At the upper end of the collimator was fixed a thallium-activated sodium-iodide crystal (Ekco, $\frac{11}{16}$ in. diameter and 1 in. thick). This was in optical contact with the face of a photo-multiplier tube. The whole was enclosed in lead shielding 5 cm thick. The photo-multiplier tube was connected to an amplifier and single channel pulse anlyser and thence to a scaler (Text-fig. 1).

In six experiments, 50–100 μc of ^{131}I-labelled human serum albumin in 0·1 ml. of isotonic saline (albumin 20 mg/100 ml.) was injected into the lateral ventricle, as above, over 1 min. Generally the grooves in which the sledge ran were blocked so that activity could be counted in either of two positions, *A* or *B*, 10 cm apart. Immediately after injection of ^{131}I-albumin, successive $\frac{1}{2}$ min counts of activity were made with the sledge alternately in

positions *A* and *B*. The counts were read visually from the scaler without its being stopped, the readings being made at the precise moments when the sledge was being moved. Thus not only could fluctuations of activity in one position be recorded, but also the relative time course of the fluctuations in two positions. At 1 hr the animal was killed with intravenous Nembutal, the head-rest was removed, a polythene bag was placed over the head to protect the collimator shielding from possible contamination, the groove-blocks were removed and 1 min counts were made at 2 cm intervals over the full length of the spinal cord. In two experiments the sledge was kept in one position over the course of the hour. In such cases, the counts were recorded at 1 min intervals. In one experiment, 1 min counts were made in ten different positions, 2 cm apart, on either side of the posterior end of the spinal cord. This was continued for 5 hr. In two control experiments the same dose of ^{131}I-albumin was injected into the cisterna magna.

Colloidal graphite. An approximately 1 % suspension of colloidal graphite (Aquadag, Acheson Colloids) was prepared in artificial c.s.f. containing 0·5 % polyvinyl alcohol. This was centrifuged at 2500 rev/min for 10 min to remove any aggregates. The makers state that most of the particles are 1 μ or below, with a maximum of 5 μ. This suspension was injected into the lateral ventricle of a prone unclamped rabbit at a rate of 0·0170 ml./min for 2 hr. At the end of this period the animal was turned into the supine position without its longitudinal axis being disturbed from the horizontal. The vascular system was perfused with 5 % formol saline through the aorta after being washed out with isotonic saline. Specimens of cord and filum terminale plus the adjacent nerve roots were then removed for sectioning and staining. The distribution of carbon particles in the sections was noted under the light microscope. Four rabbits were studied in this manner.

Other species. Simple experiments to determine initially whether or not Evans Blue entered the central canal of the spinal cord from the ventricular system were performed in the following species: the rhesus monkey (*Macaca rhesus*), the cat (*Felis catus*), the rat (*Rattus norvegicus*), the guinea-pig (*Cavia porcellus*), and the sea-lamprey (*Petromyzon marinus*). In each species except the lamprey the animal was anaesthetized, laid prone with its head slightly raised, and a small-volume injection of Evans Blue made into its lateral ventricle. The procedures were similar to those used in the rabbit. Anaesthesia was maintained for 2 hr with the animal lying in the same position, but without any clamping of its vertebral column. At the end of this period the animal was killed by an injection of Nembutal. The skull and vertebral column were dissected from the rest of the body with a minimum of disturbance and were transferred, without their longitudinal axis being displaced from the horizontal, into the freezing mixture. The column was sectioned at 2 cm intervals in a similar manner to that of the rabbit. Details are given below of the number of individuals of each species studied, the method of anaesthesia, site of ventricular puncture and volume and concentration of Evans Blue injected.

Rhesus monkey (3). Nembutal, intravenous; 1·1 cm posterior to coronal suture, 0·9 cm lateral to mid line, 2·0 cm deep; 0·3 ml. of 2 % Evans Blue.

Cat (2). Nembutal, intraperitoneal; 1·5 cm posterior to coronal suture, 1·0 cm lateral to mid line, 1·2 cm deep; 0·4 ml. of 2 % Evans Blue.

Rat (3). Open ether; 0·9 cm posterior to posterior border of orbit, 0·5 cm from mid line and 0·3 cm deep; 0·025 ml. of 2 % Evans Blue.

Guinea pig (2). Open ether; 0·6 cm posterior to coronal suture, 0·55 cm lateral to mid line, 0·2 mm deep; 0·05 ml. of 1 % Evans Blue.

Sea-lampreys (5). These were obtained from the river Severn near Tewkesbury during late May. They enter fresh water to spawn at that time of year. They were transported to London in well-oxygenated tap-water cooled to about 10° C. They were kept for up to 14 days in well-circulated and oxygenated water at 13° C. Under these conditions they remained healthy without fatality. Anaesthesia was begun by placing a lamprey in water containing 1:10,000 tricaine (MS-222, Sandoz). After about 10 min the lamprey became limp and motionless, except for its respiratory movements. It was removed from the water and

polythene tubing (4 mm *o.d.*) was inserted through the mouth into its branchial chamber. Water containing a similar or rather lower concentration of tricaine was allowed to flow into the branchial chamber and passed out readily through the gill-openings. In this way anaesthesia could be maintained for at least 30 min without cessation of respiratory movements and with complete recovery of the lamprey on its return to tricaine-free water.

The anaesthetized lamprey was fixed with its long axis straight and horizontal by means of a series of clamps, lined with foam plastic. A fine needle (20 gauge) was inserted vertically downwards with a rack and pinion, exactly in the mid line, 0·5 cm posterior to the posterior border of the transparent tissue above the pineal eye. This places the needle tip in the IVth ventricle into which 0·04 ml. of 1 % Evans Blue was injected. The needle was withdrawn and the lamprey returned to anaesthetic-free water for 2 hr. At the end of this period it was killed with tricaine and the brain and spinal cord dissected out.

In two other anaesthetized lampreys, the muscle and cartilage overlying the anterior end of the C.N.S. was dissected away and the brain and anterior 5 cm of the cord, both covered with perimeningeal tissue and primitive meninx, exposed. With care these coverings and the choroid roof of the IVth ventricle could be removed without bleeding occurring into the ventricle. A drop of 1 % Evans Blue was then inserted directly into the IVth ventricle and the semi-transparent anterior end of the spinal cord observed to assess any progress of Evans Blue along the central canal.

Finally, the complete brain and anterior end of the spinal cord were dissected and removed from two anaesthetized lampreys. These were placed intact in 0·9 % NaCl on a microscope slide. A drop of colloidal graphite suspension was placed in the IVth ventricle. With a cover-slip in position the movements of the particles within the ventricle and entrance of the central canal were observed under the light microscope.

RESULTS

Evans Blue in the rabbit

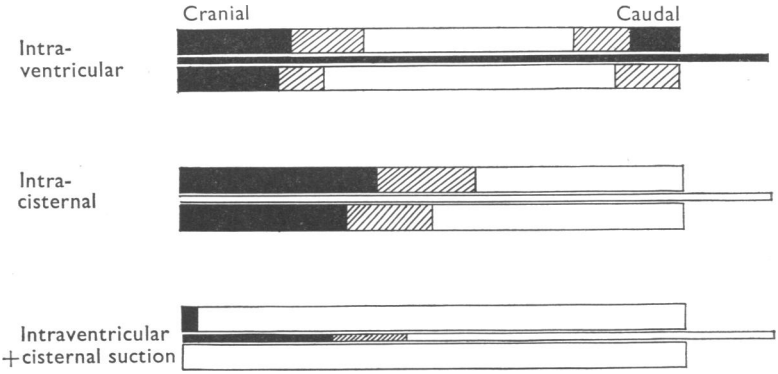
The final distribution of Evans Blue relative to the spinal cord, following intraventricular and intracisternal injection, is shown diagrammatically in Text-fig. 2.

Intraventricular injection. After injection into the ventricle, dye was present throughout the ventricular system of the brain in high concentration except in the non-injected lateral ventricle, which was generally only lightly coloured. The c.s.f. in the cisterna magna and basal cisterns contained much dye, and after 2 hr coloration was extending upwards and around the olfactory lobes to involve the anterior part of the upper surface of the frontal lobes, up the central sulci and up and around the external surface of the mid-brain to meet in the mid line and to involve the anterior surface of the cerebellum behind the tentorium and the posterior surfaces of the occipital lobes in front of the tentorium.

The spread of dye in the subarachnoid space of the cord from the cisterna magna was limited in all experiments and barely reached two fifths of the way along the outside of the spinal cord. The spread was somewhat greater on the dorsal surface of the cord than on its ventral surface.

The central canal of the cord was filled with Evans Blue at all levels, and the blue coloration extended into the filum terminale. Dye was

present in the spaces between the sacral nerve roots, the highest concentration occurring closest to the filum terminale. The dorsal surface of the caudal end of the cord was blue for a few centimetres forward of the filum terminale. This escape of dye into the lumbosacral space appears to occur through the walls of the filum terminale, since the cut surfaces of the frozen blocks containing the caudal end of the cord always showed uncoloured nervous tissue between the central canal and the pia mater. There was always a large part of the spinal subarachnoid space which contained no dye between the dye front originating from the cisterna magna and that extending in a cranial direction from the filum terminale.



Text-fig. 2. Final distribution of dye at 2 hr following intraventricular injection of 0·2 ml. of 1 % Evans Blue (five rabbits), intracisternal injection (three rabbits) and intraventricular injection with a negative pressure of -15 cm H_2O maintained at the cisterna magna (two rabbits). For each type of experiment, top horizontal column refers to dorsal subarachnoid distribution, middle column to distribution in central canal and filum terminale and bottom column to ventral subarachnoid distribution. Blacked rectangles indicate marked coloration and cross-hatched, some coloration visible. Distances as fractions of total spinal cord length.

In two experiments (Text-fig. 2), continuous drainage of c.s.f. from the cisterna through a needle at a negative pressure of -15 cm water (Davson, Pollay & Purvis, 1962) was established 5 min before the injection of 0·2 ml. of 1 % Evans Blue into the lateral ventricle. Two hours later the only part of the subarachnoid system that contained Evans Blue was that immediately adjacent to the foramina of Luschka & Magendie, i.e. within about 1 cm of these openings. The ventricular system was coloured as in the other intraventricular injection experiments, but dye had only penetrated about half the length of the central canal.

Intracisternal injection. After injection into the cisterna magna, the distribution of dye on the external surface of the brain was similar to that following intraventricular injection, but there was no reflux of dye into

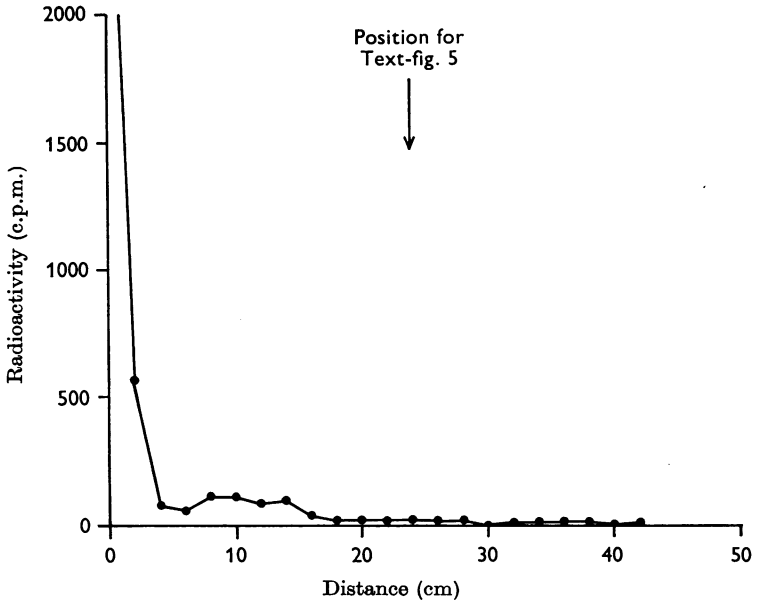
any part of the ventricular system, the ependyma of the IVth ventricle being quite uncoloured. There was a greater spread of dye in the spinal subarachnoid space than that following intraventricular injection, which no doubt reflects the fact that immediately after intracisternal injection the highest concentration of dye in the c.s.f. was further caudal. Even under these circumstances, however, the spread of dye in the spinal subarachnoid space seldom extended more than half-way along the cord. As in the intraventricular experiments the concentration of dye was somewhat greater dorsally than ventrally (Text-fig. 2).

In great contrast to the ventricular experiments, there was no penetration of dye into the central canal, no doubt in association with the lack of dye in the IVth ventricle, nor was there dye in the filum terminale, in the subarachnoid space adjacent to the lumbosacral cord, or around the sacral nerve roots.

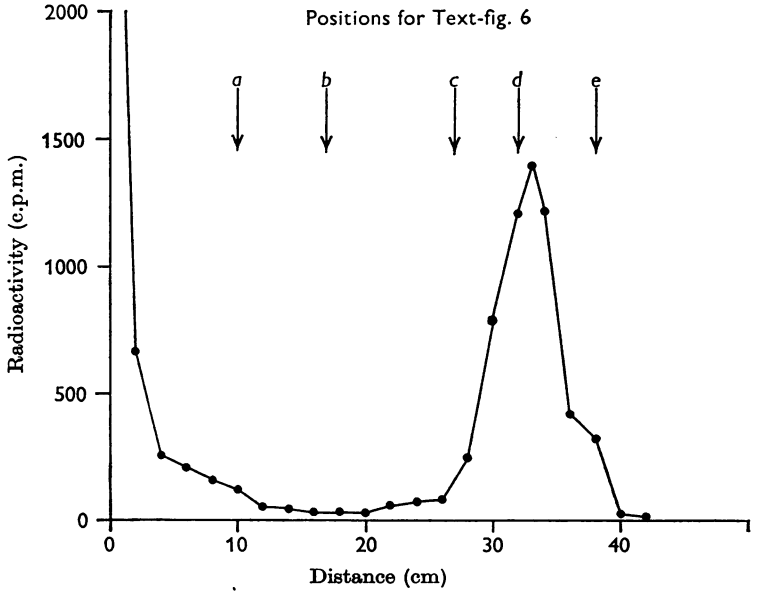
^{131}I-albumin in the rabbit

Text-figures 3 and 4 indicate the spatial distribution of radioactivity over the vertebral canal at 1 hr after the injection of ^{131}I-albumin into the cisterna magna and the lateral ventricle respectively. After intracisternal injection there was much activity over the occipital region of the skull, which diminished steeply more posteriorly over the cervical part of the vertebral column and at 18 cm from the posterior end of the IVth ventricle was only slightly above background. There was no peak of activity over the lumbosacral region. When the collimator was over a point 24 cm from the origin, the activity rose slowly to about 20 c.p.m. above background during the first hour after injection. It is reasonable to suppose that the greatest concentration of radioactive material was still in the region of the cisterna magna at 1 hr and that the moderate activity extending to 18 cm is due to ^{131}I-albumin in the cervical and anterior thoracic subarachnoid space. The slow rise, just above background, posterior to this is probably due to drainage of c.s.f., containing ^{131}I-activity, into the blood stream.

After intraventricular injection (Text-fig. 4) the picture over the cisterna magna and the anterior end of the vertebral canal was similar, except that the moderate activity, attributed to tailward subarachnoid spread, did not extend quite as far as after intracisternal injection, as was the case after injection of Evans Blue in these two ways. More posteriorly, however, there was a large peak of activity over the lumbosacral region. The maximum height of this peak occurred at the point where the spinal cord merges into the filum terminale, i.e. at the vertebral spine of S 2. Since this peak was not present after intracisternal injection, it must be caused by ^{131}I-albumin which has passed posteriorly along the central canal of the cord and has escaped or is in the process of escaping into the sacral subarachnoid space.

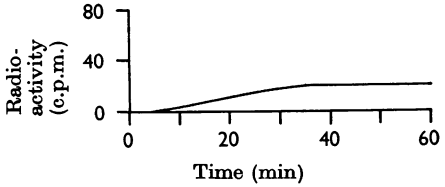


Text-fig. 3. Radioactivity (counts per min) over the spinal cord at different distances from the posterior angle of the IVth ventricle at 1 hr after the injection of 100 μc of ^{131}I-albumin into the cisterna magna.



Text-fig. 4. Radioactivity (c.p.m.) over the spinal cord at different distances from the posterior angle of the IVth ventricle at 1 hr after the injection of 100 μc of ^{131}I-albumin into the lateral ventricle.

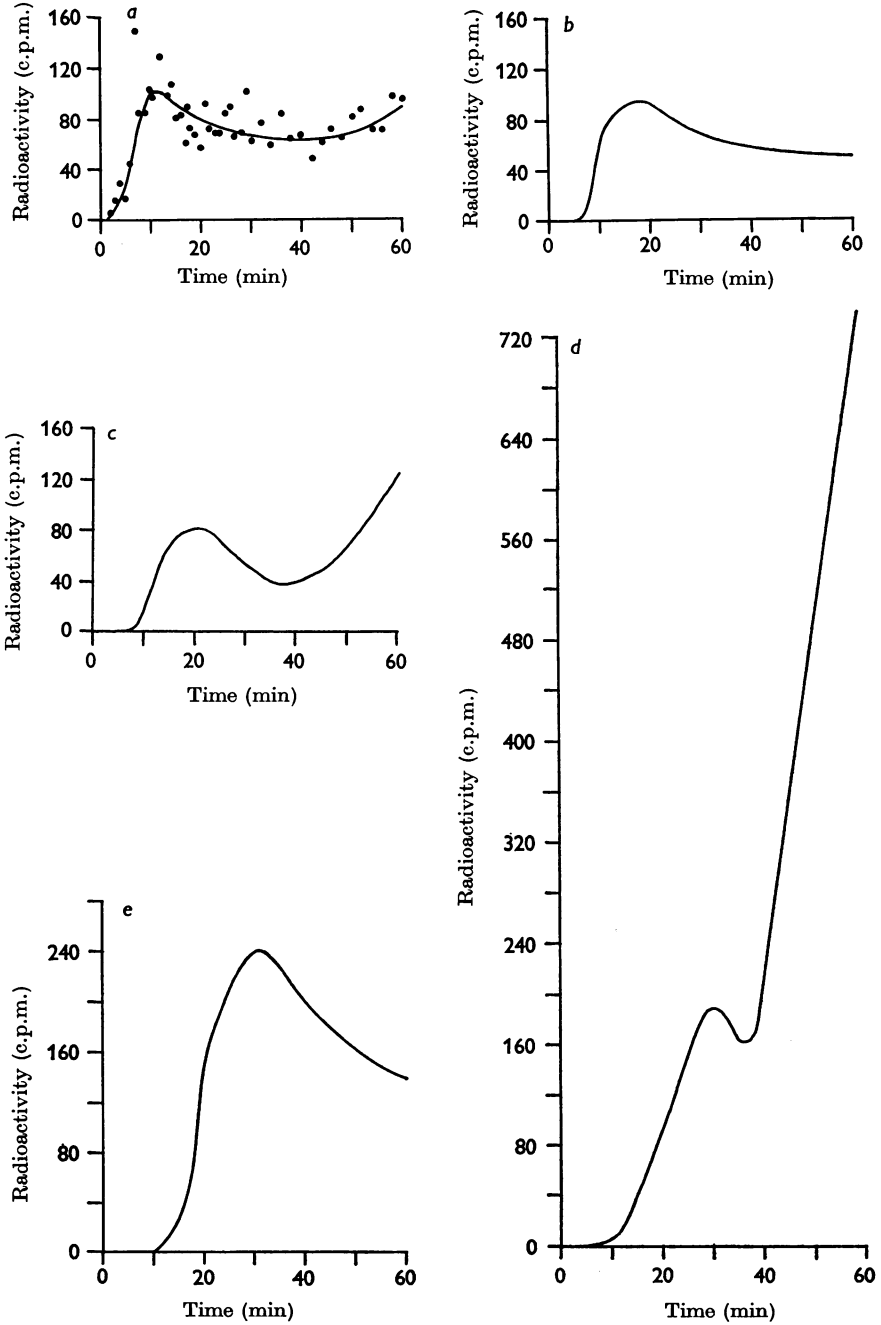
Since external counting showed the accumulation of activity at the lumbosacral end of the vertebral column so well, activity over different parts of the cord was recorded continuously during the first hour after the injection of ^{131}I-albumin, in the hope that a component due to radioactive c.s.f. actually in the central canal might be detected. Text-figures 5 and 6*a–e* depict the results of such experiments made with the collimator at different distances from the origin. In positions at which there was little or no subarachnoid activity (Text-fig. 6*a–c*), the count rate was always low and hence there was much chance variation between individual ½ min or 1 min counts. Nevertheless, the main trends in activity could be easily



Text-fig. 5. Radioactivity (c.p.m.) over a point on the spinal cord 24 cm from the posterior angle of the IVth ventricle (see Text-fig. 3) at different times after the injection of 100 μc of ^{131}I-albumin into the cisterna magna.

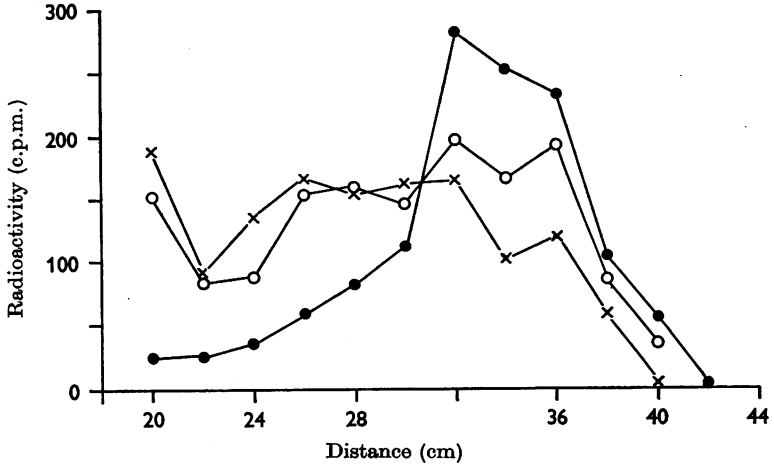
followed and the rising phase of the curves was always distinct. It is probable that the single small peak or first small peak in Text-fig. 6*a–d* is due to activity actually in the canal. Initially in each case there will be activity in the canal; at injection the ventricular c.s.f. becomes heavily labelled, and as this heavily labelled c.s.f. flows caudally along the canal, it causes a steep rise in activity when its front reaches the collimator. The further the collimator is from the origin, the later does this rise occur. During the course of the hour, heavily labelled ventricular c.s.f. becomes diluted with newly formed non-radioactive c.s.f. and the activity in the ventricular system and secondarily in the canal falls (Text-fig. 6*a–d*). Where subarachnoid activity is accumulating by spread from the anterior subarachnoid space (Text-fig. 6*a*), or by escape from the filum terminale (*c–e*), there is a secondary rise in activity which, when the collimator is close to, or at, the point of escape, is large.

If the central canal wave of radioactivity was recorded alternately at ½ min intervals in the two positions, 10 cm apart, the steep rising phases of the two peaks were quite distinct. The rising phase of the peak recorded in the posterior position always occurred a few minutes after the same phase recorded in the anterior position. In three such experiments, the mean rate of advance of the front of radioactivity was 0·34 ± 0·08 mm/sec. The area of cross-section of the central canal is about 0·025 mm^2, so the rate of flow of c.s.f. along the central canal must be of the order of 0·5 μl./min or about 1/20 of the total c.s.f. production.



Text-fig. 6*a–e*. Radioactivity (c.p.m.) over points on the spinal cord 6, 17, 27, 32 and 38 cm from the posterior angle of the IVth ventricle (see Text-fig. 4) at different times after the injection of 100 μc of ^{131}I-albumin into the lateral ventricle. Experiments on different animals.

Since c.s.f. from the central canal is being continually added to the pool of c.s.f. in the lumbosacral subarachnoid space, there must be a corresponding net loss of c.s.f. from this site if equilibrium is to be maintained. The rate of turnover, however, may be expected to be slow, since the rate of addition of c.s.f. from the central canal is small compared to the presumed volume of c.s.f. in the lumbosacral subarachnoid space. Either c.s.f. must escape from the space locally into adjacent blood vessels, lymphatics or tissues, or there must be a slow cranially directed net flow of spinal subarachnoid c.s.f. In an attempt to gain information on these



Text-fig. 7. Radioactivity (c.p.m.) over the lumbosacral spinal cord and filum terminale at different distances from the posterior angle of the IVth ventricle at 1 hr (●), 2½ hr (○) and 4 hr (×) after the injection of 100 μc of ^{131}I-albumin into the lateral ventricle.

points, the shape of the lumbosacral peak of activity was recorded in one rabbit over 4 hr (Text-fig. 7). The peak becomes progressively flattened and spreads forwards over this period. There is no marked diminution in the total activity present and at 2½ and 4 hr there is evidence that the front of activity, arising from the cisterna magna, is spreading caudally in the subarachnoid space. The impression is that continuous slow mixing, occurring throughout the subarachnoid space, tends to mask any evidence of local escape of radioactivity or of net flow of c.s.f. in a cranial or caudal direction.

Colloidal graphite in the rabbit

After intraventricular injection of colloidal graphite, macroscopic examination of the brain and spinal cord revealed that the carbon particles penetrate into the same regions as intraventricularly administered Evans

Blue. Notably, blackening of the central canal, filum terminale and lumbosacral subarachnoid space occurred.

Under the microscope, longitudinal sections of the region, where spinal cord merges into the filum terminale and nerve cells are sparsely scattered in a connective tissue matrix (Pl. 1, fig. 1), showed carbon particles in the central canal from which arise blind ependyma-lined diverticula. There are aberrant patches of ependymal cells within the matrix of the cord. Carbon was present in the subarachnoid space.

In the filum terminale (Pl. 1, fig. 2) the central canal breaks up into an irregular maze of channels, mostly lined by ependyma, and there are wide open connexions with the subarachnoid space into which carbon could be seen passing from the central canal or its branches.

Beyond the region where the canal connects with the subarachnoid space, the filum terminale (Pl. 1, fig. 3) continues as an irregular meshwork of ependymal cells. Carbon was still visible occasionally in spaces between ependymal cells and was present in the subarachnoid space between the filum terminale and the sacral nerve roots.

Other species

In none of the other species investigated, namely the rhesus monkey, the cat, the rat, and guinea-pig and the sea-lamprey, was any appreciable penetration of Evans Blue into the central canal noted after its intraventricular injection. Generally, blue staining extended no further than the point where the IVth ventricle joins the central canal. In one guinea-pig out of two the Evans Blue penetrated some 3 cm caudally along the central canal. In two rats out of three, the full length of the central canal was coloured, but only very faintly, whereas the ventricular system was heavily stained. The faint blue dot in the centre of the cut surfaces of the frozen segments of cord, representing the central canal, contrasted markedly with the deep blue dot in the centre of all regions invariably seen in the rabbit's spinal cord after intraventricular injection.

After the open introduction of Evans Blue into the IVth ventricle of the sea-lamprey, the dye could be watched penetrating the proximal end of the central canal as a blue streak. This streak never extended more than 3 mm from the end of the ventricle. A similar degree of penetration was observed in the cord of one lamprey after Evans Blue was introduced directly into the IVth ventricle through a needle without dissection.

Observation of carbon particles within the IVth ventricle under the light microscope confirmed that a marked cilia-mediated circulation of c.s.f. occurs within the ventricular system itself. In the floor of the ventricle there was a fast and narrow stream of particles passing caudally in the ventral groove. When this stream reached the V-shaped posterior

end of the ventricle it was deflected upwards and laterally and the particles returned towards the head in a slower, broader stream which lay above and on either side of the ventral fast narrow caudally directed stream. No entry of particles into the canal for more than 3 mm was noted, but dissection of pieces of cord from different regions in a drop of saline revealed some ciliary activity on the ependymal cells of the central canal.

DISCUSSION

The experiments with Evans Blue show that c.s.f. flows caudally along the central canal of the rabbit. That flow occurs under ordinary circumstances, as well as under the experimental conditions employed, is indicated by the following considerations. The injection of 0·2 ml. of 1% Evans Blue into one lateral ventricle was insufficient to cause a detectable rise of pressure in the ventricular system and indeed full penetration of the canal was observed after the injection of as little as 0·05 ml. Hence, it is unlikely that penetration resulted artifactually from the elevation of hydrostatic pressure, attending the injection of fluid into the lateral ventricle. Presumably there must be a transient small rise in ventricular pressure, but even if this could account for some of the spread of dye, it is difficult to see how it could solely be responsible for preferential flow in the canal, when compared with that in the spinal subarachnoid space. Furthermore, the experiments in which ^{131}I-albumin was used argue against transient changes in hydrostatic pressure as a cause of the observed flow in the central canal. Increase of activity was still occurring over the lumbo-sacral region at 1 hr after the injection of ^{131}I-albumin into the ventricle (Text-fig. 6*d*). By this time any transient difference of hydrostatic pressure between the ventricular system and the lumbosacral subarachnoid space must have subsided.

The experiments in which Evans Blue or ^{131}I-albumin were injected into the ventricular system also lead to the conclusion that the c.s.f., which flows along the central canal, escapes into the sacral subarachnoid space. The presence of a caudally directed flow presupposes such a fate for the c.s.f. in the canal anyway, as there is nowhere else for it to go, except for the possibility of its being absorbed into the cord itself through the ependyma lining the canal. The experiments in which dye or labelled protein was injected into the cisterna magna prove indisputably that these substances reach the lumbosacral region in the ventricular experiments from the canal and not through the spinal subarachnoid space. They eliminate any question of the results being due to preferential staining of the pia in this region or preferential uptake of radioactivity by the pia. Proof of the existence of pathways suitable for the bulk flow of c.s.f. from the canal into

the subarachnoid space is yielded by the sections obtained after infusion of colloidal graphite into the ventricle; these show carbon-filled channels communicating between the inside and the outside of the filum terminale.

The mechanism of movement of c.s.f. along the central canal was not established in these experiments. However, the estimated velocity of flow of 0·34 mm/sec from experiments in which ^{131}I-albumin was used is in good agreement with the rate of movement of particles across the ciliated surfaces of isolated frog oesophagus and rabbit trachea (Hill, 1957). At the respective body temperatures of the frog and rabbit, these rates are similar and vary between 0·3 and 0·5 mm/sec. In addition, cilia protruding from the ependyma of the central canal of the rabbit have been seen with the electron microscope (Luse, 1956). Thus it seems probable that cilia provide the motive power for the flow demonstrated in the canal.

It is improbable that this flow fulfills any essential function for the rabbit, since the central nervous systems of several mammals apparently work very successfully without it; nor does it seem likely that the rabbit has developed such a specialized feature in isolation. The alternative is to suppose that it is a relic of vertebrate evolutionary history, which has disappeared from most species but happens to have persisted in the rabbit; or else, similarly, it is a feature of the early developmental stages of the individual vertebrate, embryo, larva, foetus, or newly born animal, which in the case of the rabbit has persisted into the adult. In either circumstance, a flow of c.s.f. along the central canal might have a transport function.

The lamprey seemed a good primitive vertebrate in which to look for a central canal flow, as not only is it known to have cilia in the central canal of its spinal cord (Schultz, Berkowitz & Pease, 1956), but no blood vessels penetrate into the substance of the nervous tissue of the cord (Kappers, Huber & Crosby, 1936; Schultz *et al.* 1956). A flow, if found, would have suggested an alternative transport system to the externally situated blood vessels. In fact, there is no evidence of such a flow in the lamprey. The negative findings in this species are thus against a canal flow being a feature of the rabbit's early adult ancestors, though they do not, of course, exclude this.

We are left with the supposition that it may be a feature of the early embryological development of some or all vertebrates, and indeed movements of cilia within the central canal of the cord of certain vertebrate larvae have been reported (Olsson, 1958). These ciliary movements in the canal of larvae appear to be associated not only with movements of the c.s.f. but with the backward progress of Reissner's fibre. This structure, of probable mucopolysaccharide nature, is secreted by the ependymal subcommissural organ and runs backward through the ventricles and

central canal before infiltrating between the cells of the extreme posterior tip of the cord into the surrounding mesenchyme (Olsson, 1955, 1958). It has no known function, nor have we evidence as to whether it exists in the adult rabbit.

On the other hand, the presence of cilia on the ventricular ependyma has been well documented (Valentin, 1842; Studnicka, 1900; Stoklasa, 1930) since the early observations of Purkinje (1836). Indeed some of those who grow glial cells in tissue culture like to include some ependymal tissue in order that the activity of its cilia may act as an indicator of the good health of the cultured cells or the suitability of a culture medium (Hild, 1958). Such studies as have been made of their effect on the movements of c.s.f. indicate that they do indeed cause streaming of c.s.f. within the ventricles of amphibia and rabbits (Chu, 1942) and that in *Xenopus* larvae (Adam, 1953) quite complex circulatory currents may result.

SUMMARY

1. A caudally directed flow of c.s.f. occurs in the central canal of the spinal cord of the rabbit.

2. C.s.f. from the canal escapes through the filum terminale into the sacral subarachnoid space. This escape appears to occur through channels, lined with an epithelium which is generally continuous on the one hand with the ependyma and on the other with the pia mater.

3. The rate of advance of a front of radioactivity in the canal was 0·34 mm/sec which indicates a flow of about 0·5 μl./min or about 1/20 of the total c.s.f. production.

4. The rate of advance and other evidence are compatible with the flow being cilia-mediated.

5. A flow of c.s.f. could not be demonstrated in the central canal of the adult sea-lamprey, the guinea-pig, the rat, the cat and the rhesus monkey. In the rabbit, it may be a relic of evolutionary history or a persistence into the adult of a feature of embryological development.

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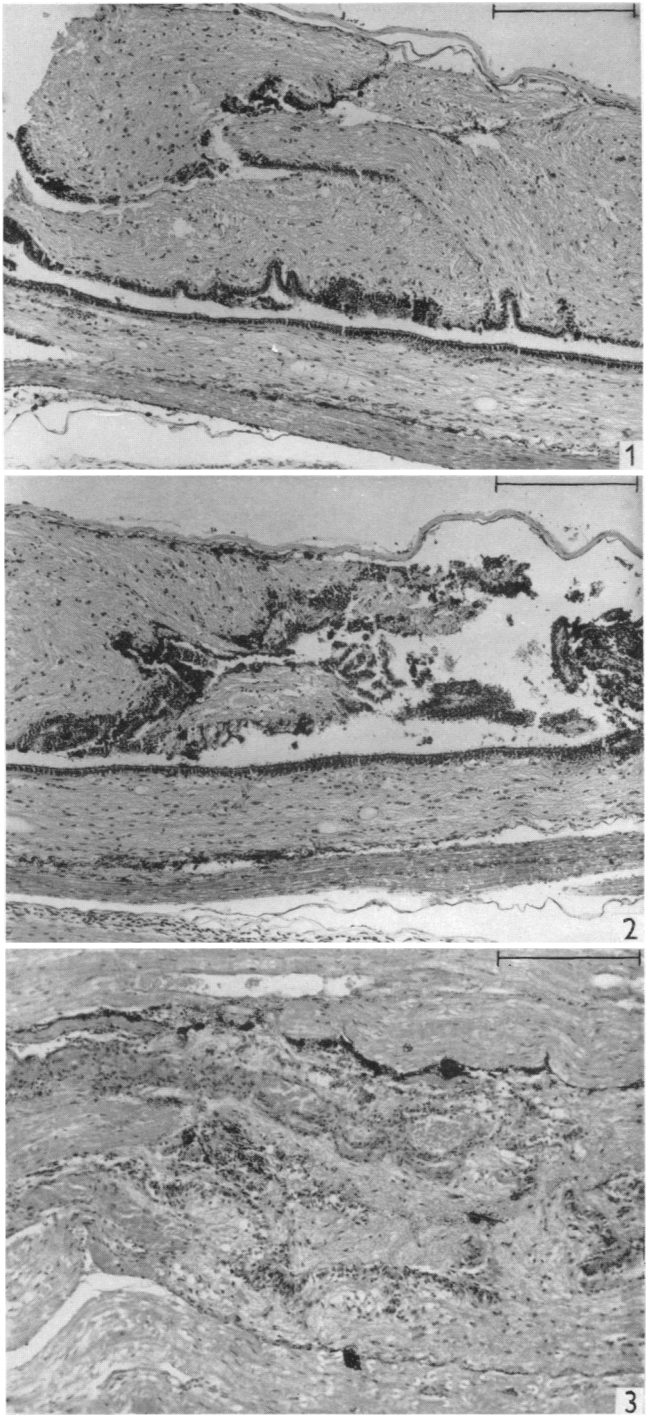
EXPLANATION OF PLATE

Longitudinal sections through the junctional region of spinal cord and filum terminale (Figs. 1 and 2) and filum terminale proper (Fig. 3) of rabbit, after the infusion of 1 % colloidal graphite into lateral ventricle at 0·0170 ml./min for 2 hr. Stained haematoxylin and eosin; thickness 10 μ; scale marks, 250 μ; orientation: cranial, left and caudal, right.

Fig. 1. Blind diverticula, lined with ependyma, are arising from the central canal; carbon is present in the central canal and subarachnoid space.

Fig. 2. Same section as Fig. 1, photomicrograph of region just caudal to that of Fig. 1. Central canal is breaking up into numerous channels, mostly lined with ependyma. Carbon is passing through wide opening into subarachnoid space.

Fig. 3. The filum terminale consists of an irregular meshwork of ependymal cells in a connective tissue matrix; carbon is present in the subarachnoid space on either side of this; outside the subarachnoid space lie sacral nerve roots.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