INVESTIGATION OF THE SUBARACHNOID SPACES OF THE BRAIN BY INJECTION OF ACTIVELY MOTILE PARTICLES (INFUSORIANS) INTO THE CEREBROSPINAL FLUID

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The structural features of the canals and cells, the communications between them, and the character of movement of the cerebrospinal fluid (CSF) in each of these systems were studied in an intravital experiment using a technique of injection of actively motile particles (infusorians) into the subarachnoid space of a dog. Communications between the canals and cells were found to be patent for infusorians in both directions. Movement of dead infusorians with the CSF is purely passive.

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Injection of passively moving particles (emulsions, suspensions, erythrocytes, etc.) into the cerebrospinal fluid (CSF) has revealed marked differences in the pattern of CSF movement in the system of canals and the system of cells in the pia mater covering the cerebral hemispheres [1-5].

The object of the present investigation was the intravital study of communications in these systems by injecting motile particles (infusorians) into the CSF. It is to be expected that movement of infusorians will be less dependent on movement of the CSF itself, but more dependent on the structural features of the subarachnoid space.

EXPERIMENTAL METHOD

Experiments were carried out on dogs weighing 10-15 kg anesthetized with morphine and thiopental. A burr-hole 14 mm in diameter was made in the parietal region and the dura beneath this hole was removed. The brain surface was irrigated with warm physiological saline. A needle connected to a manometer (as described by F. M. Lyass) was introduced into the cisterna magna, enabling the CSF pressure to be measured dynamically and maintained throughout the experiment at 15-20 mm water, and also used for injecting the suspension of infusorians and solution of trypan blue into the cisterna magna and for the withdrawal of samples from it.

Preliminary experiments in vitro showed that if infusorians (Paramoecium caudatum) are kept for several hours in normal CSF their movements are not disturbed. The infusorians were cultivated on an infusion of hay, stained intravitally in it with neutral red (1 : 50,000), washed by centrifugation in water, and then injected into the cisterna magna. Movement of the infusorians in the subarachnoid spaces was observed in a stereoscopic microscope in incident light under magnifications of between 25 and 87.5 times. In another modification the infusorians were injected directly into the lumen of a canal or into the region of the cells by means of a thin needle. At the conclusion of the experiments, 2 ml of a 0.2% solution of trypan blue was injected into the cisterna.

EXPERIMENTAL RESULTS

From 1 to 2 min after injection of the infusorians into the cisterna magna they appeared in the canals crossing the gyri of the parietal lobe. Because of the bright red color of the digestive vacuoles, they stood out clearly against the white background of the brain (Fig. 1).

In fixed specimens the canals resembled endothelial tubes with arteries inside their lumen. For considerable distances the walls of the canals contained orifices leading into cells. On intravital observa-

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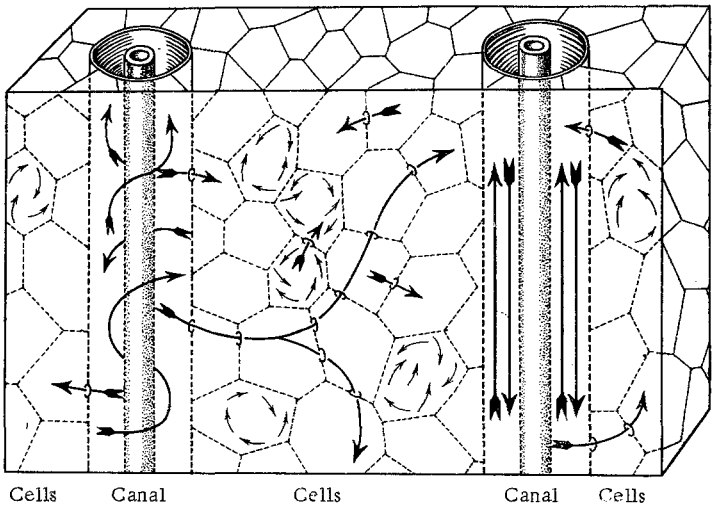

Fig. 1. Scheme of movement of infusorians in subarachnoid space of cerebral hemispheres. Cells are located between two canals with arteries running in them. Thin arrows in canal on left indicate direction of spontaneous movement of infusorians in canal space and their escape through certain places in the canal wall into surrounding cells. Thick arrows in canal on right show direction of oscillating movements of infusorians. Arrows in zone of cells show movement of infusorians through certain points into adjacent cells and trajectory of their movement through a long series of cells. Small arrows show irregular movement of infusorians inside individual cells.

tions the walls of the canals and orifices in them could not be distinguished. The only guide to the canals was the position of the arteries, along the course of which the infusorians, injected into the CSF, were spread. Nevertheless, movement of the infusorians clearly outlined the invisible wall of the canals. When they touched this wall the infusorians changed direction and did not go outside the canal.

In the canals the infusorians combined their spontaneous movement with the oscillating movement of the CSF. The spontaneous movement took place in different directions along the lumen of the canal: the infusorians described circles and spirals around the artery, moved from one wall of the canal to the other, moved along the canal, and so on. Since each infusorian is covered by about 2500 cilia, they move at considerable speed. Puslatory, and even more marked respiratory oscillations of the CSF had no effect on movement of the infusorians in the fully anesthetized animal. Only when the animals awoke from the anesthetic and gave a muscular contraction, a forced respiration, or changed the position of its body were the powerful oscillatory currents of CSF, much greater in amplitude than the respiratory and pulsatory oscillations, sufficient to affect the infusorians and carry them along the canals into the cisterns at the base of the brain, after which they returned along the same path to the surface of the cerebral hemispheres. Spontaneous movement of the infusorians was then resumed in the canals.

The whole space on the gyri of the hemispheres located between adjacent canals (arteries) is occupied by cells. Intravital observations likewise fail to reveal cell walls in this region, or the orifices in these walls, which are clearly visible in histological specimens. As already mentioned, infusorians initially appeared in the canals only, and over the greater part of their course did not escape into the cells. Later, places were found in the wall of the canals through which the infusorians penetrated into the cells and where, consequently, orifices were present connecting the canals with the cells.

Movement of the infusorians in the cell zone showed a number of distinctive features.

1. No oscillatory movements of infusorians were observed there in the course of muscular contractions, forced respiration, or changes in the body position. Whereas in the canals oscillatory currents of CSF swept the infusorians in opposite directions alternately, in the cells their movement was entirely

spontaneous. 2. In contract to suspensions, erythrocytes, and similar particles, infusorians were not immobilized and were not trapped in the cells, thanks to the activity of their cilia. 3. The overwhelming majority of infusorians penetrating into the cells moved irregularly in small, clearly defined spaces. On striking the wall of the cells they turned and described a curve inside these spaces. However, some infusorians, having escaped through certain points in the cell walls, resumed their irregular movement in neighboring small spaces. Here also, at these points, were communications connecting adjacent cells. 4. In cases when a large number of infusorians arrived along a canal, many of them could be seen to escape through certain points in the canal wall and to move through a series of cells along a definite trajectory. These infusorians moved in single file like a row of trucks, readily visible against the background of the irregular movement of the great majority of infusorians.

In the other modification of the experiments, infusorians were injected through a fine needle directly into canals or cells. In the first case the picture of mass escape of infusorians through definite places in the canal wall into the cells was observed. In the second case, when infusorians were injected into the cells, they moved along them revealing the position of communications between the cells. Under these conditions, because of the large number of infusorians in the cells, movement of some of them could be detected in the direction of the canals, followed by escape into their lumen. The infusorians could thus pass through communications between the canals and cells in either direction.

In the final stage of the experiment a solution of trypan blue was injected into the cisterna magna. This dye selectively stains the stellate collagen structures which mark the junction between the walls of adjacent cells, thus greatly increasing the accuracy of intravital observations of the topography of the two pial systems. During the 7-10 min after injection of trypan blue, the pattern of movement of the infusorians as described above continued to be observed, in this case with exceptional clarity. Staining of the "stars" made movement of the infusorians along the constant trajectories through a long series of cells particularly demonstrative. Later, however, the dye began to have a toxic effect and the infusorians were immobilized. Dead infusorians moved with the CSF like "floats" and their movement was now indistinguishable from that of suspensions, erythrocytes, and so on. In the canals they gave only oscillatory movements: pulsatory, respiratory movements and movements synchronized with muscular contractions, which interferred with each other. In the cells all the infusorians remained motionless. Microscopic examination of CSF withdrawn from the cisterna magna showed that dead infusorians were carried suspended in the CSF in the canals. So far as the infusorians trapped in the cells are concerned, they could not be withdrawn by this method. They remained firmly fixed in the cells and did not escape into the canals.

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