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The injection of phenosulfonphthalein (PSP) into the lateral ventricles is followed, usually within minutes, by the appearance of the dye in the cerebrospinal fluid (CSF) of the basilar cisterns and subarachnoid space. Failure of the dye to appear, or any substantial delay in its rate of appearance, may be regarded as strong evidence of an obstruction of the ventricular system. On this basis, the dye has been widely used in the clinical diagnosis of hydrocephalus and has served to distinguish the obstructive and communicating types reliably.\(^1\to7\)

In the current experiment, the circulation of PSP in the ventricles and subarachnoid space of normal and hydrocephalic animals was investigated. A number of observations concerning the normal migration of the dye as well as its altered circulation in obstructive and communicating hydrocephalus have not been previously reported. The significance of these findings is discussed.

Materials and Methods

Rhesus monkeys (macaca mulatta) and purebred beagle dogs were used in this study. The monkeys ranged in age from 1\(^\frac{3}{4}\) to 2 years and varied in weight from 4 to 6 lbs. The monkeys were ostensibly well at the time of the experiment. The dogs, on the other hand, were chosen from a line of purebred beagles in which a high incidence of communicating hydrocephalus spontaneously occurs.\(^6\) The dogs ranged in age from 2 days to 4 years and weighed between 500 gm and 40 lbs. For purposes of this experiment the animals were separated into three groups:

**Group 1 (Normal).** This group was composed of normal rhesus monkeys and normal beagle dogs. In the dogs, pneumoencephalograms had been previously performed to separate the normal animals from those with hydrocephalus. All findings were later confirmed by pathological examination.

**Group 2 (Communicating Hydrocephalus).** This group was composed of beagle dogs in which severe hydrocephalus had been previously demonstrated by pneumoencephalography and, in some cases, by Pantopaque ventriculography. The communicating nature of the process was suggested by the lack of air over the convexities and by the free passage of Pantopaque from the lateral ventricles into the cisterna magna. The diagnosis was later substantiated by pathological study.

**Group 3 (Obstructive Hydrocephalus).** Obstructive hydrocephalus was produced in a number of rhesus monkeys by blocking the fourth ventricle and distal aqueduct with an inflatable balloon. This technique has been discussed elsewhere\(^16\) and causes advanced ventricular enlargement in a matter of hours.\(^17\) Experiments were performed on these animals at intervals between 1 hour and 14 days after obstruction of the ventricular system.

In approximately half of the animals of each group, 0.2 cc of PSP\(^*\) was injected into one lateral ventricle through a 23 gauge nee-

\(^*\)In the current experiment, the widely available form of PSP (U.S.P., ph 6.2–7.0) was used. Dandy’s original work was done with “neutral PSP” (Dandy’s solution, ph 6.0–6.5) which is no longer commercially available.
dye; in the other half a similar dose was injected into the cisterna magna. The animals were sacrificed at intervals ranging from 45 minutes to 5 hours after injection of the dye and perfused with 10% neutral formalin solution via the aortic arch for 45 minutes. Thereafter, the brains were carefully removed and immersed in 1.0 N sodium hydroxide (NaOH) solution to develop the dye which becomes pink when alkalinized. Immediate color photographs were taken to document the findings, and routine sections were saved for microscopic study.

Results

Group 1 (Normal): Ventricular Injection of PSP. At 45 minutes after injection of PSP into the lateral ventricles of normal monkeys and dogs, the dye was found diffusely distributed throughout the subarachnoid space (Fig. 1). The leptomeninges of all the major cisterns were deeply stained as were the leptomeninges over the cerebral and cerebellar hemispheres and the leptomeninges of the spinal subarachnoid space. Migration of the dye into the depths of the cortical sulci was evident on coronal section of these brains.

Of particular interest was the finding that PSP leaves the ventricles and subarachnoid space with apparent ease and penetrates the surrounding brain to a depth of several millimeters (Fig. 1 right). The dye was found in the cortical ribbon of gray matter over the hemispheres and was particularly intense at the base of the sulci. Little or no dye was present in the subcortical white matter. Around the ventricles, the dye was observed in a number of periventricular structures. Here, too, the gray matter was selectively stained with a rich uptake in the following structures: the periaqueductal gray, the hypothalamus, the caudate nucleus, and the thalamic nuclei bordering the third ventricle. The septum pellucidum was another richly stained structure, but the midline commissures and other periventricular structures of white matter origin were minimally stained or not stained at all. Examination of brains 2 hours after intraventricular injection of PSP revealed no qualitative differences from the findings at 45 minutes although the migration of dye into the surrounding brain was somewhat increased.

Group 1 (Normal): Cisternal Injection of PSP. Injection of PSP into the cisterna magna of normal animals was followed within 45 minutes by diffuse spread of the dye throughout the subarachnoid space. The pattern of this distribution and the penetration into the surrounding brain were similar to that seen after intraventricular injection. The dye did not enter the ventricular system by retrograde flow, and although the outlets of the fourth ventricle were vitally stained, the cavity of the ventricle contained no dye.

Group 2 (Communicating Hydrocephalus): Ventricular Injection of PSP. Following injection of PSP into the lateral ventricles of dogs with communicating hydrocephalus, the dye was found to pass promptly out of the ventricles into the surrounding cisterns. Within 45 minutes, the leptomeninges of the spinal subarachnoid space were intensely stained as were the leptomeninges of the basilar cisterns, the ambient cisterns, cisterna magna cerebri, and the interpeduncular cisterna. Dye did not pass over the cerebral convexities but was heavily concentrated over the cerebellar hemispheres where it penetrated the sulcal grooves between the folia. Only a small trace of dye passed into the prechiasmatic and Sylvian fissure cisterns. From these findings it was presumed that the obstruction was the result of obliteration of the subarachnoid spaces over the cerebral hemispheres. This finding was subsequently supported by microscopic findings, which demonstrated a diffuse, non-specific leptomeningitis, most advanced over the cerebral hemispheres.

On coronal section of these brains, it was apparent that a considerable transependymal migration of dye had occurred. The depth of the parenchymal penetration and the intensity of the vital staining were substantially greater than that observed in the normal controls. In contrast to the normal controls, the dye in the hydrocephalic brains had migrated into periventricular structures of both white and gray matter origin, although the latter was preferentially stained. Surface staining of the cerebellum, brain stem, and spinal cord was also evident to a depth of 4 to 5 mm, but no dye was seen in the parenchyma over the cerebral hemispheres owing
to the limited migration of dye above the level of the tentorium.

**Group 2 (Communicating Hydrocephalus): Cisternal Injection of PSP.** When PSP was injected into the cisterna magna of dogs with communicating hydrocephalus, the dye migrated throughout the subarachnoid space until its cephalad flow was halted at the level of the obstruction. In addition, however, the dye was found to have migrated along a retrograde route into the ventricular system and to have distributed itself throughout all four ventricles. This, of course, was in great variance with the findings in the normal controls, in which no such retrograde flow occurred. Furthermore, a rich staining of periventricular structures was evident and was of the order of that seen after intraventricular injection.

**Group 3 (Obstructive Hydrocephalus): Ventricular Injection of PSP.** The alteration of PSP circulation in obstructive hydrocephalus was of particular interest. As expected, PSP introduced into the lateral ventricles failed to pass into the basilar cisterns or subarachnoid space as long as 5 hours after injection of the dye. This indicated that the block was essentially complete and ruled out the existence of spontaneous ventriculostomies.

On coronal section of these brains, considerable extraventricular migration of dye was noted (Fig. 2). In some areas the dye penetrated the brain to a depth of 5 to 6 mm; in other areas the penetration was considerably deeper. This migration was substantially greater than that seen in the brains of normal controls, and the extent of the staining was far more pronounced. As noted in the brains with communicating hydrocephalus, the migration of dye out of the ventricles was not confined to an uptake in structures of gray matter origin. In addition to a rich staining of the hypothalamus, caudate nucleus, periaqueductal gray, septum pellucidum, and periventricular thalamic nuclei, a more moderate migration of dye had occurred into the following structures: the corpus callosum, the columns of the fornix, the midline commissures, and the white matter bordering the lateral ventricles. Less intense but distinct staining was seen in the area of the centrum ovale.

**Group 3 (Obstructive Hydrocephalus): Cisternal Injection of PSP.** When PSP was injected into the cisterna magna of animals with obstructive hydrocephalus, the dye was observed to distribute throughout the spinal subarachnoid space and basilar cisterns up to the level of the incisura of the tentorium. A migration of dye into the surface cisterns over the cerebellum and into the ambient cisterns was also evident but there was a complete obstruction to migration beyond this point (Fig. 3). No dye was observed above the level of the tentorium, and the picture was that of an incisural block. It was difficult to determine whether this obstruction was the consequence of obliteration of the cerebral subarachnoid spaces from compression and flattening of the cerebral gyri, the consequence of expansion of the cerebral hemispheres with a choking off of the tentorial notch, or the result of both. The gross and microscopic pathological data, however, suggested that both phenomena occur.

On coronal section of the brains it was clear that no dye had entered the ventricular system and that except for a penetration of 2 to 3 mm into the surface of the cerebellum, brain stem, and spinal cord, no dye had migrated into the brain parenchyma.

**Discussion**

There is now substantial evidence\textsuperscript{5,12} that once CSF (and presumably PSP) leaves the lateral ventricles, the direction of flow occurs in the following sequence: cephalad from the cisterna magna into the premedullary, preoptic, and cerebellopontine angle cisterns, around the brain stem to the ambient cisterns and cisterna vena magna cerebri, and forward into the interpeduncular and prechiasmatic cisterns. From all these cisterns the flow is then directed into an intricate system of subarachnoid pathways that invest the cerebral, cerebellar, and spinal cord surfaces and descend into the depth of the cortical sulci.

**Normal Findings.** In the current experiment, the migration of PSP following intraventricular or cisternal injection into normal dogs and monkeys was found to distribute
FIG. 1. Normal brains 45 minutes after intraventricular injection of PSP. Note diffuse distribution throughout the subarachnoid space including that over the cerebellar and cerebral hemispheres. In coronal section (right) note that dye has migrated across ependymal and pia-glial surfaces to penetrate gray matter to a depth of several millimeters. The choroid plexus is richly stained.

FIG. 2. Monkey brain with obstructive hydrocephalus 45 minutes after intraventricular injection of PSP. Coronal section shows that dye has migrated into the brain to a considerable extent. Note involvement of white and gray matter structures and the absence of dye in the subarachnoid space.

FIG. 3. Monkey brain with obstructive hydrocephalus 2 hours after cisternal injection of PSP. Note the heavy concentration of dye over the cerebellum and the lack of PSP migration above the level of the tentorium.
along expected CSF flow routes. Of interest was the finding that the dye migrates across ependymal and pia-glial boundaries to penetrate the brain with apparent ease. In 1909 Goldmann\textsuperscript{11} demonstrated that trypan blue injected into the CSF spreads quickly throughout the fluid and penetrates the surrounding parenchyma to a limited extent. In 1960, Feldberg and Fleischhauer\textsuperscript{10} noted a similar transependymal escape of bromophenol blue when the dye was injected into the lateral ventricles. Although these dyes are classified as colloidal dyes and therefore differ from PSP which is soluble in water, the migration of the different dyes appears to be essentially the same.

The mechanism by which PSP penetrates the cerebral parenchyma is not known. There is a growing body of evidence, however, to suggest that the ependymal and pia-glial surfaces are more permeable than previously believed and that they do not stand as a barrier to a number of substances including proteins. Thus, Bowsher\textsuperscript{3} and Lee and Olszewski\textsuperscript{15} demonstrated that labelled proteins pass rapidly out of the lateral ventricles to penetrate the surrounding brain. Similarly, Brightman\textsuperscript{4} has shown a free passage of ferritin and peroxidase between the epithelial cells of the choroid plexus. This intercellular migration is to be distinguished from the pinocytotic inclusion, which occurs in ependymal cells\textsuperscript{15} and also in glial and neuronal processes.\textsuperscript{4} It is also to be distinguished from the phagocytosis of particulate matter by the mesothelial lining cells, which behave like macrophages and engulf foreign substances in an active manner.\textsuperscript{9,18}

From what is known about CSF-brain and brain-blood barriers, it is unlikely that the migration of PSP across ependymal and pia-glial boundaries is accompanied by much, if any, absorption of the dye. The current findings emphasize, however, the essential permeability of these surfaces and suggest that at least a limited flow of CSF occurs across these membranes by diffusion. The fate of PSP and other CSF constituents, once within the cerebral parenchyma, is uncertain. Whereas it is probable that PSP is removed by pinocytosis by the glial and neuronal processes, it is equally probable that certain components of CSF such as electrolytes, water, and even proteins are absorbed and removed across the brain-blood barrier. Whether this transependymal and pia-glial circulation represents a significant pathway for CSF absorption (in addition to the pathway across the arachnoid villi) is a subject of considerable interest.

The selective uptake of PSP by structures of gray matter origin has been reported. It is unlikely that local differences in ependymal and pia-glial permeability account for this finding. Either the gray matter has a greater capacity for including the dye or a lesser capacity for releasing it. Quite possibly, the dye is removed more rapidly from white matter so that the increased concentration in gray matter is a spurious observation.

\textbf{Findings in Communicating Hydrocephalus.} The finding that PSP passes readily out of the ventricles and migrates throughout the subarachnoid space until the obstruction is reached is consistent with the usual findings of pneumoencephalography and isotope cisternography in patients with communicating hydrocephalus. Of greater interest was the finding that accompanying this decrease in subarachnoid circulation there was an increased flow of dye out of the ventricles, across ependymal surfaces, into the surrounding brain. Quite possibly, the pathological changes that accompany ventricular dilatation, namely, stretching of the ependymal surface with disruption of intercellular junctions and frank damage to the cells themselves, produce an avenue for increased flow out of the ventricles. It is also quite possible that the increased intraventricular pressures that develop in hydrocephalus contribute to the flow. An important question is whether the increased flow out of the ventricles is associated with increased absorption. If this be so, as Bering and Sato\textsuperscript{2} have suggested, then the alteration may serve as an equilibratory factor in hydrocephalus.

The migration of PSP into the cerebral ventricles following cisternal injection was of considerable interest. This reversal of flow may simply reflect a generalized mixing that occurs in a closed but pulsatile fluid system, or it may imply a more extensive alteration of circulatory mechanisms including the little-known function of the ependymal cilia.

\textbf{Findings in Obstructive Hydrocephalus.} When the block in obstructive hydrocephalus is essentially complete, there is no migra-
tion of dye out of the ventricles and into the surrounding subarachnoid space. In the current experiment, however, transependymal flow was found to be substantially increased. It is unlikely that this deep and rapid penetration can be explained by phagocytosis or by increased absorption of both dye and CSF is raised. Clinically, it is not uncommon to find some urinary excretion of PSP in cases of obstructive hydrocephalus in which the obstruction is believed to be complete. It is possible, therefore, as Laurence suggested in 1959, that transependymal and periventricular absorption accounts for this finding.

Finally, when PSP was injected into the cisterna magna of animals with complete obstructive hydrocephalus, the dye was found to halt abruptly in its cephalad migration at the incisura of the tentorium. This suggests that in severe obstructive hydrocephalus a secondary obstruction of the subarachnoid space may develop. On the basis of the current findings, it is probable that this is the consequence of two pathological changes: 1) obliteration of the cerebral subarachnoid space by compression and flattening of the gyri; and 2) expansion of the cerebral hemispheres from ventricular dilation, with choking of the tentorial notch. Another possibility is that the absence of CSF flow out of the ventricles retards the migration of dye in the subarachnoid space. Further data will be necessary before this point can be resolved.

The foregoing observations may be of some practical importance. In patients with obstructive hydrocephalus, for example, it is well known that apparently ideal operations such as third ventriculostomy and ventriculocisternostomy (the Torkildsen shunt) are much less effective than might be expected. Quite possibly, an alteration of the absorptive surfaces over the convexities accounts for this failure, even when the process is an acquired one. In cases of congenital hydrocephalus, on the other hand, it is quite possible that the potential subarachnoid space over the convexities is compromised in utero, accounting for the lack of development of these spaces often noted at necropsy.

**Summary**

The flow of phenosulfonphthalein (PSP) in monkeys and dogs was studied under normal and hydrocephalic conditions. The following observations were made:

1. The circulation of PSP in cerebrospinal fluid is determined by the direction of the CSF flow.
2. Under normal conditions, PSP passes quickly out of the lateral ventricles to reach the cisterna magna, surrounding cisterns, and subarachnoid space. The dye crosses ependymal and pia-glial surfaces with apparent ease and penetrates the surrounding brain to a depth of several millimeters.
3. In communicating hydrocephalus, PSP passes readily out of the ventricles and migrates throughout the subarachnoid space until the extraventricular block is reached. In addition, a substantial transependymal migration occurs which is considerably greater than under normal conditions. When the dye is injected into the cisterna magna, it migrates both in a cephalad direction to the point of obstruction and along a retrograde route to enter the ventricles.
4. In obstructive hydrocephalus, there is no migration of dye out of the ventricles and into the surrounding subarachnoid space. Transependymal migration of dye is greatly increased, however, and the dye can be detected in the depths of the white matter within 2 hours. When dye is injected into the cisterna magna, it is abruptly halted in its cephalad migration at the incisura of the tentorium. This suggests that in severe obstructive hydrocephalus a secondary obstruction of the subarachnoid space may develop.
5. Whether transependymal and pia-glial migration of dye represents a lesser pathway for CSF absorption, and whether this pathway becomes more important in hydrocephalus, are questions yet to be determined.

**Bibliography**

2. Bering, E. A., Jr., and Sato, O. Hydrocephalus: changes in formation and absorption