Resumen por el autor, L. H. Weed.

La absorción del líquido cerebro-espinal en el sistema venoso.

El presente trabajo ha sido llevado a cabo con el control fisiológico adecuado sobre las presiones del líquido cerebro-espinal y las de los sistemas arterial y venoso intracraniales. El curso seguido por la absorción del liquido cerebro-espinal bajo condiciones normales es a través de las vellosidades de la aracnoides, penetrando en los senos venosos durales. Bajo la influencia de un aumento en el contenido salino de la sangre, producido por la inyección intravenosa de una solución fuertemente hipertónica (ClNa al 30 por ciento), la absorción tiene lugar también por medio de los canales perivasculares y a través del epitelio ependimario que tapiza los ventrículos cerebrales, hasta llegar a los capilares del sistema nervioso. En el proceso normal la filtración puede ser el factor físico de mayor importancia, pero después de la inyección intravenosa de soluciones hipertónicas muy fuertes, la ósmosis y la difusión juegan aparentemente el papel más activo. Bajo condiciones normales no tiene lugar la absorción de materia particulada desde el espacio subaracnoideo y lo mismo sucede después de la inyección intravenosa de una solución fuertemente hipertónica.

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THE ABSORPTION OF CEREBROSPINAL FLUID INTO THE VENOUS SYSTEM

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TWO FIGURES AND FOUR CHARTS

With but few exceptions, all recent investigators of the processes of the cerebrospinal fluid have agreed that by far the major portion of the fluid is absorbed directly into the venous system and that only a very small portion escapes by way of lymphatic channels. Many of the observations upon which this conception is based have been of a physiological nature; the anatomical studies, fewer in number, have established in fairly definite fashion the exact pathways taken by this fluid in its return to the blood stream. The general mechanisms, both anatomical and physiological, have thus come to be quite well understood though in many details uncertainty still exists. But now, because of more accurate technical procedures and because of certain physiological phenomenon reported during the past three years, new approaches to the investigation of the general subject of the venous absorption of the cerebrospinal fluid may be employed and it becomes possible to control, in more effective fashion, the experimental work.

The writer in his first work on the absorption of this fluid ('14 a, b, c). made use of procedures which permitted control of many of the physiological factors concerned. In place of the customary anatomical method of injecting under high pressures viscous colloidal solutions or fatty substances into cadavers or dead animals, an isotonic, true solution of potassium ferrocyanide and iron-ammonium citrate was introduced into the subarachnoid space of living animals; the foreign salts were subsequently precipitated in situ as Prussian blue for histological study. In

these experiments the injection of the foreign solution was maintained at levels slightly above the normal pressure of the cerebrospinal fluid but no record of the venous pressures within the cranium was made. At this time, nine years ago, the procedures for ascertaining the intracranial venous pressures were not adequate, but now, the simple method of obtaining the pressure in the superior sagittal sinus as it empties into the torcular Herophili, reported by Hughson and the writer ('21 b), renders it possible to determine accurately these pressures. The importance of recording cerebral venous pressure in investigations of the cerebrospinal fluid has been emphasized by many workers in this field and with this standard it becomes possible to establish the physiological conditions under which an anatomical investigation of the absorption of the cerebrospinal fluid is undertaken.

In addition to this control of intracranial vascular pressures, the present study has extended the brief investigations of McKibben and the writer ('19 b) regarding the dislocation of the cerebrospinal fluid into the nervous system following intravenous injections of strongly hypertonic solutions. This aspiration of fluid from the subarachnoid space by way of the perivascular channels into the nervous tissue, under the extraordinary osmotic pull of the blood, suggested that the mechanism of absorption of the cerebrospinal fluid might in this way be altered: the usual process of filtration of the cerebrospinal fluid into the venous system, might, under the conditions of experimentation, be replaced by a process in which osmosis and diffusion were the determining factors.

With such possibilities in view, the investigation on which the present paper is based was undertaken. With the physiological factors apparently adequately controlled, the process of absorption of the cerebrospinal fluid into the venous system was again studied anatomically; the general findings coincide with the conclusions previously presented, but it has been possible to make the scope of the investigation more comprehensive and to control more satisfactorily the experimental procedures than in the previous work.

REVIEW OF IMPORTANT THEORIES OF ABSORPTION

It is not purposed to give in detail the various theories advanced regarding the absorption of the cerebrospinal fluid, as extensive reviews of the literature of the subject have been presented elsewhere (Key and Retzius, '76; Weed, '14 a, '22). But with the problem definitely enlarged by the methods of adequate physiological control and by the pressure-alterations effected by intravenous hypertonic solutions, it seems possible to gain additional information regarding the correctness of the theories proposed. To this end, the important hypotheses concerning the pathways of absorption of the cerebrospinal fluid will be briefly recorded.

The basic anatomical work done on this subject was that carried out by Key and Retzius ('76) and reported by them in a superb monograph. The anatomical pathway of absorption of the cerebrospinal fluid was studied by means of injection, under relatively high pressures (60 mm. Hg), of a colored gelatine mass into the spinal subarachnoid space of cadavers. The essential pathway of absorption of the fluid was found to be by way of the Pacchionian granulations into the great dural venous sinuses. In addition to this major mechanism, an accessory drainage indirectly into the lymphatic system was demonstrated.

For many years after this publication, the ideas of Key and Retzius prevailed but with the realization that Pacchionian granulations as such do not exist in infants and in the higher mammals, the view came to be considered inadequate. In the decade from 1890 to 1900, many investigators (Reiner and Schnitzler '94, Hill '96, Lewandowsky '00, and others) demonstrated by physiological methods that there occurred a rapid absorption of the cerebrospinal fluid into the venous system of living animals, with an accessory, far slower passage into lymphatics. And in the last twelve years renewed interest in the anatomical pathway of absorption has become manifest. Mott's ('10) important conception of the process hypothesized the passage of the cerebrospinal fluid from subarachnoid space through the perivascular channels into the cerebral capillaries. This hypothesis was based on the remarkable dilatation of the peri-

vascular, pericapillary and perineuronal spaces in the brains of animals which had been subjected to experimental cerebral anemia by ligation of carotid or vertebral arteries. Mott's studies were entirely histological but they gave convincing microscopic evidence of the existence of potential pathways from nerve-cell and cerebral capillary to subarachnoid space.

Shortly after the publication of Mott's hypothesis, Dandy and Blackfan ('13, '14) reported the results of their investigations of this problem, basing their conclusions largely upon the excretion of a soluble dye—phenolsulphonephthalein—after introduction into the subarachnoid space. Their only anatomical studies were made on animals in which India ink was injected into the subarachnoid space; there was no absorption of these insoluble carbon particles—an observation in accord with those of Quincke ('72) and of Sicard and Cestan ('04). Dandy and Blackfan interpreted their findings with phenolsulphonephthalein to mean that the absorption of cerebrospinal fluid was "a diffuse process from the entire subarachnoid space." Subsequently Dandy ('19) pointed out that the cranial portion of this space was by far more efficient than the spinal.

The writer's earlier work ('14 a, b, c) on this mechanism of absorption was published a short time after the initial publication of Dandy and Blackfan. Subarachnoid injections were made in living animals over periods of several hours' duration, under pressures but slightly in excess of the normal (130 to 180 mm. H_2O). The findings after such injection of India ink were quite similar to those of the other workers in the field; apparently only a minimal passage of the carbon particles outward from the subarachnoid space occurred. When, however, the problem was studied by means of subarachnoid injections of isotonic solutions of potassium ferrocyanide and iron-ammonium citrate (a procedure which permitted histological identification of the pathway taken by the foreign solution), it was found that the major absorption of the fluid was directly into the great dural venous sinuses by way of arachnoid villi. These villi were found to occur in all of the common laboratory mammals and in infants; when hypertrophied, as in adult life and in old age,

they were identified as the well-known Pacchionian granulations. With an accessory indirect drainage of the cerebrospinal fluid into the lymphatic system also indicated, these observations of the writer coincided largely with those of Key and Retzius, though based on a standard of experimentation which more nearly approximated the normal.

EXPERIMENTAL PROCEDURES

The methods of investigation in this present study depended essentially upon adequate physiological control of the experimentation. To this end, the anatomical injections were all made on living animals (dog,, cat) with simultaneous records of all of the important pressures. Without special preliminary preparations, the animals were anesthetized with ether as this anesthetic has been found to give constant levels of the pressure of the cerebrospinal fluid (Weed and McKibben '19 a, Weed and Hughson '21 a). After etherization of the animal by cone an intratracheal tube was introduced and the anesthetic administered throughout the rest of the experiment by Woulfe bottle. Systemic venous pressure was recorded by insertion of a suitable cannula in the superficial brachial vein and connection of this cannula with an open-end manometer containing Ringer's solution. The superior sagittal sinus was exposed by rongeuring away a narrow gutter in the mid-line of the calvarium, taking care to avoid injury to the dura mater. A cannula was inserted into one of the common carotid arteries and connected to a mercury U-manometer; the intracarotid pressure recorded gave, as is well known, a qualitative record of the intracranial arterial pressure. The subarachnoid space was then entered by puncture with suitable needles through the occipito-atlantoid ligament or between the lumbar arches. With the loss of as little cerebrospinal fluid as possible, the puncture-needle was connected to an open-end manometer of 1 mm. bore, affording continuous record of the pressure of the fluid. The next technical procedure consisted in the insertion, posteriorly, of a needle into the superior sagittal sinus to the torcular Herophili; this

needle was then attached to an open-end manometer containing a 4 per cent solution of sodium citrate. These technical procedures have been described in detail by Weed and Hughson ('21 b) and afford accurate records of the pressure of the cerebrospinal fluid, of the intracranial and systemic venous systems, and of the intracranial arteries.

With the experiment so set up, the anatomical procedures were then instituted. After an initial control period of 5 to 10 minutes, to determine the normal levels of the pressures recorded, subarachnoid injections of two types were made. For the first or control type, combined lumbar and occipito-atlantoid punctures were made into the subarachnoid space; the foreign injection-solution was then irrigated between the two needles with release of the fluid at the needle not used for the injection. The release of the foreign solution in proper measure permitted the maintenance of the normal, preexisting pressure of the cerebrospinal fluid in the individual animal. In this way a replacement of the cerebrospinal fluid in the spinal subarachnoid space was effected without alteration of any of the normal pressure-relations, thus establishing adequate physiological control of the experimental procedure. The observations were carried out for various lengths of time, the customary period of replacement being approximately of one hour's duration.

In the second type of injection made, the experiment was so arranged that the effect of intravenous injections of strongly hypertonic solutions upon the process of absorption of the cerebrospinal fluid could be studied. The technical procedures were similar to those used for the control observations except that a cannula was inserted for the intravenous injections and a three-way cock placed between the occipito-atlantoid needle and the recording U-manometer. Connected with this cock was a burette, so arranged that the foreign solution could be introduced into the subarachnoid space without disturbing the continuous record of the pressure of the cerebrospinal fluid. Under these conditions, after the initial control period, the intravenous injection of the strongly hypertonic solution was given. Then, after 20 to 30 minutes, at which time the reduction of the cerebrospinal fluid pressure was approaching the maximum, the foreign solution was injected slowly into the subarachnoid space. This introduction of the foreign solution, slowly and in small amount, was customarily carried out over a period of from 20 to 30 minutes, and from 3 to 10 cc. of the solution were introduced. In many experiments the injection of this amount of solution was accomplished without raising the pressure of the cerebrospinal fluid above zero; in no experiment was the pressure in the subarachnoid space permitted to exceed the normal level for the individual animal. After the period of introduction of the foreign solution, the observations were usually continued for some minutes in order to ascertain whether the pressure of the cerebrospinal fluid would again fall.

Two types of foreign injections were made, both in the control and experimental observations. The first type of injection-fluid used was diluted India ink,—carbon particles of a recognizable size in suspension. This proved to be, when diluted with Ringer's solution, non-toxic in the amount used. Contrasted with this suspension of particulate matter was the second type of injection in which a true solution of foreign salts was introduced. The foreign salts employed were potassium (or sodium) ferrocyanide and iron-ammonium citrate in equal amounts; for the routine injection these salts were made up in an isotonic solution (0.5 gm. each of the foreign salts in 100 cc. of distilled water) but in a number of experiments a hypertonic solution was employed. Fixation of the tissue in an acid medium resulted in the precipitation of the foreign salts as ferric ferrocyanide (Prussian blue), which remained unaltered by the technical procedures employed for histological sections.

On completion of the experimental injections the animals were killed and fixing fluid was immediately injected through the aorta. Animals receiving the particulate suspension were injected with 10 per cent formalin alone while those given the ferrocyanide-citrate mixture were injected with 10 per cent formalin plus 2 per cent hydrochloric acid. In both groups, the central nervous system, with the dura intact, was removed two hours later and the fixation continued by immersion in

10 per cent formalin. After 24 to 48 hours, the central nervous system enclosed in its meninges was studied in gross and blocks were removed for histological study.

EXPERIMENTAL FINDINGS

The experimental observations upon the process of absorption of the cerebrospinal fluid may be divided into two groups: first, the control observations in which the cerebrospinal fluid was replaced in the spinal subarachnoid space by the foreign solution; and second, the experiments carried out with introduction of the foreign solution after the pressure of the cerebrospinal fluid had been markedly lowered by the intravenous injection of strongly hypertonic solutions of sodium chloride. The findings in these two types of experiment were quite different; the description of the results may therefore appropriately be given under two headings.

REPLACEMENT OF CEREBROSPINAL FLUID. With the experiment set up as indicated, a control-period of from 5 to 10 minutes was carried out to obtain the normal relations of the different pressures recorded. After this period, the foreign solution was run slowly into the subarachnoid space, through either the lumbar or occipito-atlantoid needle with release of the excess fluid at the other needle. In this way the spinal subarachnoid space was filled with the foreign solution (either a true solution or suspension of granules) under pressures identical with those of the cerebrospinal fluid during the control-period.

True solutions. A typical experimental record of these pressures during the initial period of control and during the period of replacement is shown in chart 1. In this animal, during the control interval, the pressure of the cerebrospinal fluid was constant at 185 mm. of Ringer's solution while the pressure in the superior sagittal sinus was but slightly above 150 mm. During the period of replacement of the cerebrospinal fluid by the isotonic solution of potassium ferrocyanide and iron-ammonium citrate, there were slight fluctuations recorded, those in the pressure of the cerebrospinal fluid being due to

technical difficulties in attaining the proper relation between the release of fluid and the introduction of the foreign solution. These changes in the pressure of the cerebrospinal fluid were reflected in similar though not identical alterations in the pressure of the superior sagittal sinus. The carotid systolic pressure dropped slightly at the beginning of the replacement, then rebounded and gradually fell during the remainder of the experimental observation.

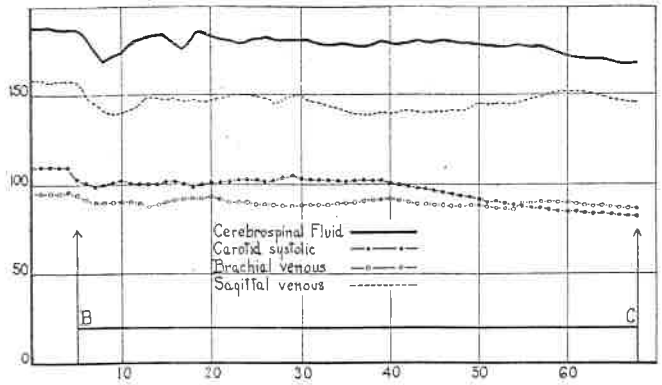

Chart 1 Experiment 22. Dog, weight 6525 grams. Ordinates represent millimeters of Ringer's solution of mercury (carotid pressure); abscissae represent time in minutes. During interval from *B* to *C*, replacement of cerebrospinal fluid in spinal subarachnoid space by isotonic solution of potassium ferrocyanide and iron-ammonium citrate.

From the anatomical standpoint, the records are of importance in demonstrating that in the experimental animal, such replacement of the spinal fluid by an isotonic foreign solution can be effected with only minimal disturbance in the essential vascular pressures. The non-toxicity of the isotonic foreign solution in the quantities injected is demonstrated by this lack of disturbance of the intracranial and systemic vascular pressures.

In post-mortem examination of animals so treated, the antemortem distribution of the foreign solution was easily made out by the resultant precipitate of the Prussian blue. The replacement of fluid between lumbar and occipito-atlantoid needles

necessarily filled only the spinal subarachnoid space; the further spread of the foreign solution was assumed to indicate the normal course of the fluid from the region of the cisterna cerebellomedullaris into which one of the needles was inserted. In the process of spread of the fluid, the normal currents of the cerebrospinal fluid and the physical phenomenon of diffusion between the normal fluid and the foreign solution play the important parts.

In such experimental animals, the subarachnoid space of the spinal cord was always densely filled with the Prussian blue granules, evidencing free communication between the irrigating needles. From the upper cervical region the granules of the blue continued into the basilar subarachnoid space about the medulla and pons and into the cisternal dilatations of the space in the region of the cerebral crura. More anteriorly the precipitation extended in the basilar spaces about the infundibulum and around the optic chiasma, to continue outward for variable distances in the subarachnoid space of the optic nerve and more restrictedly about the olfactory nerves. Such a distribution of the precipitate was obtained in all the animals even though the replacement had been carried on but a few minutes; it was assumed therefore that the initial spread of the isotonic foreign solution into the basilar cisternal dilatations of the subarachnoid space was rapid and almost immediate. In animals in which the replacement had continued for one hour, the foreign solution extended upwards from the basilar regions, usually rather completely covering the more ventral portions of the cerebellum and filling quite thoroughly the subarachnoid space in the temporofrontal angle and the Sylvian fissure. The complete injection of the subarachnoid space over the cerebral hemispheres, in such experimental replacements, required several hours, demonstrating that the currents in this portion of the space were very sluggish.

On gross inspection of the central nervous system of these animals, the dura mater was found to contain none of the blue precipitate. This freedom from coloration indicated that the foreign solution had not entered the dura during the experimental

procedure; this condition held not only for the dura mater over the cerebral hemispheres but also over the irrigated area of the spinal cord where the concentration of the foreign salts was maximal. The inner surface of the dura mater was likewise entirely free from the precipitate of Prussian blue as was also the outer surface of the arachnoid. Such findings demonstrated clearly the function of the arachnoid membrane in preventing the passage of fluid outward into the subdural space.

When the brain and spinal cord were sectioned, the precipitate of Prussian blue was found to be wholly localized within the subarachnoid space; the nervous tissue appeared absolutely free from bluish coloration even in its most peripheral zones. This lack of penetration of the nervous tissue by the isotonic foreign solution existed not only in the higher portions of the brain stem but also in the spinal cord. Such confinement of the blue precipitate to the meshes of the subarachnoid space was the most striking characteristic of the gross examination of these specimens.

The microscopic findings in these animals were in every way confirmatory of the gross distribution of the precipitated material. Sections of all regions of the spinal cord, enclosed within its three meninges, revealed an entire absence of the blue granules in the dura but a rather dense collection within the subarachnoid space. In this latter situation, the blue precipitate was seen partially filling the meshes of the arachnoidea (shown diagrammatically in fig. 1) and adhering everywhere to the cells lining the inner surface of the arachnoid membrane, covering the arachnoid trabeculae, and forming the outer surface of the pia mater. In no animal, in which the fixation was prompt and adequate, was there evidence of penetration of the lining cells of the subarachnoid space by the foreign salts; such a finding indicates therefore that the spread of the foreign solution may be assumed to represent the pathway of the cerebrospinal fluid and not merely a diffusion of the salts through the tissues. Because of the barrier offered by these lining cells of the subarachnoid space to the foreign salts, the core of the arachnoid trabeculae and the stroma of the arachnoid membrane were entirely free

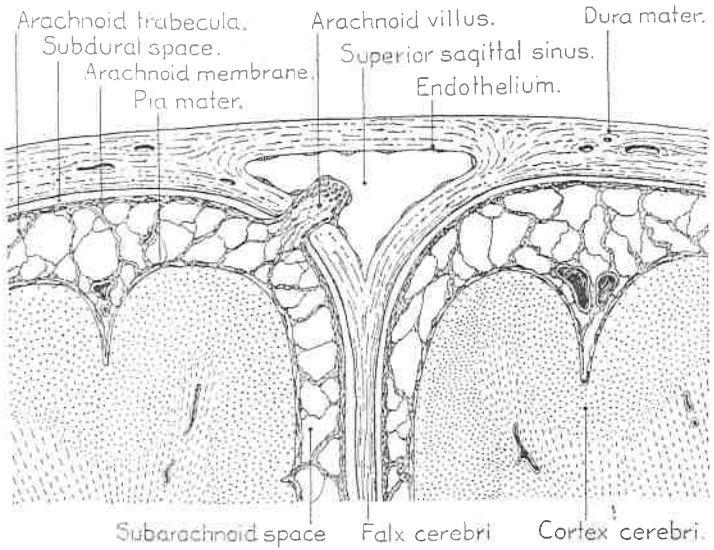

Fig. 1 Schematic diagram of coronal section of meninges and cerebral cortex, to show relation of arachnoid villus to dural venous sinus. The potential subdural space is necessarily shown of greater size than is normal; the subarachnoid space over the convolutions is also increased in width to illustrate the character of the subarachnoid mesh.

from the deposits of precipitated granules. This characteristic of the lining cells also prevented the passage of the foreign solution diffusely into the nervous tissue through the pia mater. Somewhat similarly, there was no penetration of the ferrocyanide-citrate solution, as evidenced by the precipitation, inward along the perivascular spaces. These channels, as shown diagrammatically in figure 2, connect directly with the subarachnoid space, the cells of the pia mater turning inward to form the outer layer of the cuff and the arachnoidal elements forming the inner covering. Under the conditions of injection, with maintenance of normal pressure relations within the subarachnoid space, these perivascular cuffs were found to be free from the granules; occasionally in the outer funnel-shaped dilatation (fig. 2) of the channel precipitated granules were identified. The evidence,

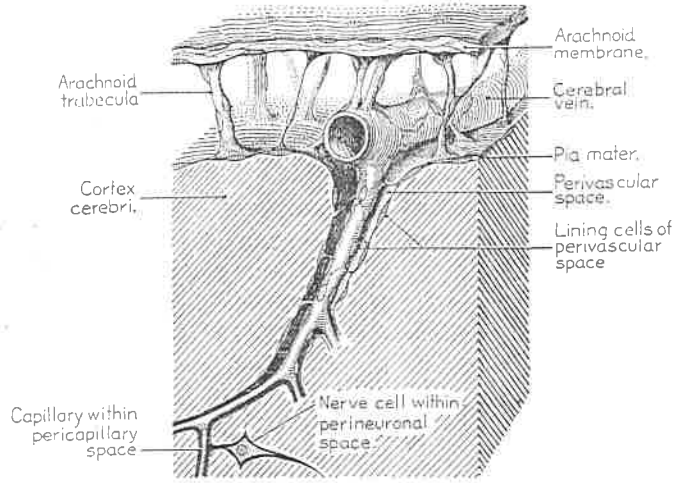

Fig. 2 Schematic diagram of leptomeninges and nervous tissue, to show relation of subarachnoid space, perivascular channels and nerve-cells. The leptomeningeal lining cells are reflected inward for various distances to form lining cells of the perivascular space.

therefore, may be taken to indicate that under conditions approaching the physiological, no passage of the fluid from subarachnoid space to nerve-cell occurs.

In the cerebral regions, a similar distribution of the precipitated material was found. The Prussian blue granules were wholly confined to the subarachnoid space, adhering to but not within the cytoplasm of the lining cells. Here again, the perivascular spaces were free from the granular material except at the dilatations adjoining the subarachnoid space. The distribution of the granules was approximately that made out in the gross inspection but the microscopic examination was convincing in demonstrating that the foreign solution had been restricted to the subarachnoid space. The granules were traced from the basilar subarachnoid space directly into the cores of arachnoid villi projecting into the basilar dural sinuses, particularly into the cavernous sinus.

The structure of the arachnoid villus, shown diagrammatically in figure 1 for those along the superior sagittal sinus, consists of a reduplication of the meshes of the arachnoidea, with usually a decreasing size of the network. The covering cells of the arachnoid membrane are carried into the defect of the dural fibrous tissue so that arachnoid cells come to lie directly beneath the endothelium of the great venous sinus. In most cases also, there is a more or less outspoken aggregation of arachnoid cells at the tip of the villus as shown in the diagram. The villi in the region of the basilar sinuses are more cellular and of a smaller mesh, but the essential structure is identical.

In the present study, the arachnoid villi are of importance in that they apparently afforded the anatomical pathway for the absorption of the cerebrospinal fluid into the venous system. In the partial injections, the precipitated granules were traced through the core of the basilar villi, through the arachnoid mesothelial cells capping the villus and through the endothelial cells of the dural sinus, directly into the lumen of the great venous channel of the base of the brain. The process appeared to be one of passage of the foreign solution directly through the cytoplasm of the cells as through a cellular membrane. In the completely injected specimens (i.e. those animals in which the experimental replacement was continued for several hours) an identical passage of the fluid, as shown by the precipitated granules, was apparent in the villi about the superior sagittal sinus.

With the evidence indicating that the foreign solution (and therefore, the cerebrospinal fluid) was absorbed directly into the dural venous sinuses by way of the arachnoid villi, an attempt was made to discover other areas of vascular absorption. The vessels traversing the subarachnoid space, those beneath the pia and those of the arachnoid membrane were found to be in no way concerned with the process; in every case the fluid was prevented from reaching the vascular channels by the barrier presented by the cells lining the subarachnoid space. No absorption by the ependymal lining of the cerebral ventricle or by the cells of the choroid plexuses was observed. Likewise no fluid was found to have passed toward the capillary bed of the nervous system by way of the perivascular spaces. Investigation of these and other possible pathways of absorption failed to reveal any escape of the fluid from the subarachnoid space into blood vessels except by way of the arachnoid villi.

When hypertonic solutions (2 to 10 per cent) of these foreign salts were used in place of the isotonic for the subarachnoid replacement, the toxicity of the salts became quickly apparent in an acceleration of the respiratory rate and in a sharp rise in intracranial arterial and venous pressures. The substitution of sodium ferrocyanide for the potassium ferrocyanide in no way affected the essential results. On anatomical examination of the nervous system of such animals, the meninges were found to be diffusely stained with the blue. The dura and arachnoid membrane showed this blue coloration and on section of the spinal cord or of the cerebral substance, a penetration of the blue precipitate inward for distances of 1 to 5 mm. was apparent. This invasion of the nervous tissue appeared, in the gross, to be diffuse, following no definite channels. Microscopically also, this diffuse penetration of the meninges and of the nervous system was made out; the cells lining the subarachnoid space appeared to offer no barrier to the passage of these hypertonic solutions. Thus, the arachnoid membrane and to a lesser extent the dura mater were completely filled with the blue granules in fine dispersion while the pia mater and the nervous tissue beneath it revealed a similar distribution of the blue granules. The mesothelial cells covering the vessels traversing the subarachnoid space, were likewise permeable to the hypertonic foreign solution and the vessel-walls (even those of rather large arteries) showed a blue granulation which extended through the endothelium. Such coloration of the vessel-walls was also observed within the perforating vessels as they traversed the perivascular cuff, and even fairly large vessels in the substance of the nervous system gave evidence of passage of the hypertonic foreign solution. These findings with the hypertonic solutions were in many ways similar to the results obtained by delay in the precipitation of the Prussian blue from the isotonic solution. In these cases, the death of the lining cells of the subarachnoid space permitted generalized and wide-spread diffusion of the foreign salts.

Granular suspensions. The experimental findings after the introduction of diluted India ink under these conditions were in many ways quite similar to those with the true solution but they gave no evidence of the essential process of absorption. The general physiological reactions to such subarachnoid replacements with India ink differed but slightly from those effected by similar replacement with the isotonic solution of potassium ferrocyanide and iron-ammonium citrate. A record of such an animal is given in chart 2, in which the replacement of the spinal subarachnoid fluid was achieved with practically no disturbance of the normal pressure-relations. The reactions of this animal may be directly compared with the data presented in chart 1.

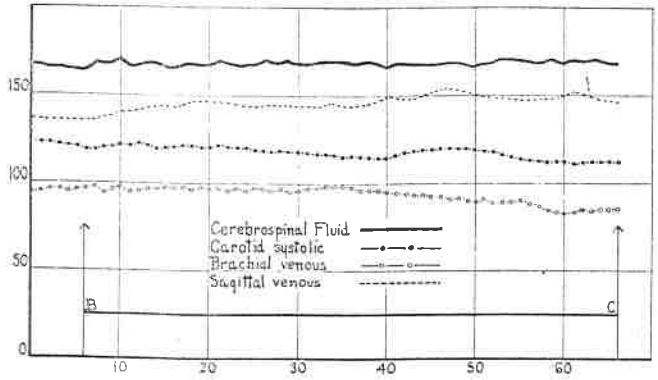

Chart 2 Experiment 23. Dog, weight 8750 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure); abscissae represent time in minutes. During interval from *B* to *C*, replacement of cerebrospinal fluid in spinal subarachnoid space by India ink, diluted 1 to 4.

The gross anatomical findings, after such a replacement with India ink, were quite similar to those detailed for the true solution. The extent of the spread of the carbon particles was roughly the same, though the subarachnoid space over the cerebrum seemed to be less rapidly and less completely filled. The carbon particles were wholly confined to the subarachnoid space, leaving the dura mater and the substance of the nervous tissue free from discoloration.

Microscopically, the general distribution of the carbon particles conformed closely to the spread of the true solution as made out histologically by the precipitate of the Prussian blue. Everywhere throughout the subarachnoid space, the particles of carbon were seen adhering to the lining cells or free in the meshes. There was no evidence of absorption of the particles nor (in the length of time of the experimental replacement), phagocytosis. The perivascular spaces were entirely free from the carbon except at the dilated connections of these channels with the subarachnoid space. No penetration of the dura mater, of the pia, nor of the arachnoid membrane could be made out; the carbon particles were wholly within the subarachnoid space. But in one essential particular, the distribution of the particulate matter differed from that of the true solution: the carbon particles were frequently massed in the arachnoid villi but in no case was there evidence of passage of the particles through the cell-membrane of the villus into the venous sinus. This finding that the granular material of a suspension was retained within the subarachnoid space is confirmatory of the observations of many workers in this field (Quincke '72, Dandy and Blackfan '13, '14, Weed '14 b, Dixon and Halliburton '16, and others).

SUBARACHNOID INJECTIONS AFTER INTRAVENOUS HYPERTONIC SALINE. This series of experiments was suggested by the profound reduction of the pressure of the cerebrospinal fluid effected by the intravenous injection of strongly hypertonic solutions, as first reported by Weed and McKibben ('19 a) and since confirmed by many other workers (Cushing and Foley '20, Foley and Putnam '20, Ebaugh and Stevenson '20, Sachs and Malone '21, Weed and Hughson '21, a, b, etc.). In their initial publication, Weed and McKibben stated that if a foreign solution of sodium ferrocyanide and iron-ammonium citrate were supplied to the subarachnoid space at the time when the pressures of the cerebrospinal fluid were approaching zero following an intravenous injection of a strongly hypertonic solution, the solution was found subsequently (p. 536) "to have passed from the subarachnoid space along the perivasculars into the substance of the nervous system, reaching the interfibrous spaces in the white

matter and the pericellular spaces in the gray." The present work is an extension of this preliminary brief study with more adequate physiological controls and with the use of both foreign true solutions and granular suspensions as the media of injection.

True solutions. The physiological reactions following the intravenous injection of strongly hypertonic solutions have been given in detail by Weed and Hughson ('21, a, b); the essential phases of these reactions are included in chart 3, which is taken from one of the current series of experiments. The effect of the intravenous injection in this animal was quite typical, with a marked reduction in the pressure of the cerebrospinal fluid to minus 72 mm. of Ringer's solution. Associated with this tremendous fall in the cerebrospinal fluid pressure were a slight rise in the arterial pressure and slight falls in both venous pressures. At the time of this drop in cerebrospinal fluid pressure to minus 72 mm., the injection of an isotonic solution of potassium ferrocyanide and iron-ammonium citrate was started; this was continued for 20 minutes, during which time 4.1 cc. was run into the subarachnoid space. The introduction of this amount of fluid caused the pressure of the cerebrospinal fluid to mount from minus 72 mm. to plus 65 mm. in this interval; the cessation of the injection was followed by a drop to 30 mm. in the next 17 minutes, showing that the hypertonic solution was still active. This rise in the pressure of the cerebrospinal fluid was accompanied by similar increases, though not of the same extent, in both brachial and sagittal venous pressures, as well as in carotid systolic pressure. Such reactions are quite typical and are contrasted with the minimal pressure-alterations following the more prolonged irrigation of the subarachnoid space; the foreign solution appeared therefore to be far more active, when given after the intravenous hypertonic solution, than alone.

On gross examination of such a specimen, after fixation by arterial injection with an acid medium, the central nervous system appeared completely surrounded by the blue precipitate. The dura mater on being removed was without blue coloration and the subdural space entirely free from any colored granules.

The subarachnoid space, in the upper one-half of the spinal cord and over the cerebral hemispheres was uniformly and densely filled with the blue precipitate. On section of the nervous tissue, the blue coloration was found to extend inward from the subarachnoid space for various distances. In the upper portion of the spinal cord, the depth of this penetration was customarily from 3 to 5 mm. while over the cerebral hemispheres it was usually somewhat less. Everywhere the coloration was densest next to the outer surface of the nervous tissue, fading rather rapidly as one passed inward. While the gross appearance was in general that of a diffuse penetration of the foreign dye, there were made out lines of denser color which ran inward. Likewise occasionally a zone of much less coloration appeared directly beneath the pia mater, interrupted, however, by the lines of denser staining running inward. The ependymal walls of all the cerebral ventricles usually showed a diffuse penetration for a depth of 1 to 4 mm., and the choroid plexuses were covered with compact blue granulation.

The microscopic findings in these specimens were very interesting. The blue precipitate, as in the replacement-experiments, was found largely within the subarachnoid space, but not penetrating any of the lining cells. These granules were traced into the arachnoid villi, where the true solution had passed through the mesothelial and endothelial cell membranes into the various dural venous sinuses. Thus there was evidence that the normal pathway of absorption of the cerebrospinal fluid was made use of under the influence of the hypertonic solution; it was only when the perivascular spaces and the ependyma were examined that additional pathways of absorption under these experimental conditions were demonstrated.

In the nervous system of all of these animals, subjected to intravenous injection of strongly hypertonic solutions and to subarachnoid introduction of the foreign solution, the perivascular spaces were found uniformly filled with the Prussian blue precipitate. These granules could be followed directly from the subarachnoid accumulations into the larger channels and into the smaller spaces about the capillaries of the nervous

system. In specimens showing penetration by the foreign solution, the nerve-cells were usually surrounded by a thin covering of granules in the perineuronal spaces which connected directly with pericapillary spaces. In addition to this aggregation of the blue precipitate about the nerve-cells, there was a more or less constant outlining of the neuroglial elements by the precipitated granules as well as a less marked, though still appreciable, generalized dispersion of the very fine blue particles throughout the white fibers and the supporting nets of the nervous system. These findings indicated that under the influence of the intravenous hypertonic solution, these potential fluid-channels of the nervous tissue were opened up and were more or less filled by the foreign solution introduced into the subarachnoid space.

Under these conditions, also, the finer blood vessels of the nervous system appeared to take part in the absorption of the cerebrospinal fluid, if one judged by the course taken by the foreign solution. In every case in which the perivascular system was well filled with the precipitated granules, the endothelial walls of the capillaries showed similar fine particles within the cytoplasm and other particles of the Prussian blue were identified lying free within the lumen of the capillary. Occasionally the smallest venules seemed from similar evidence to be playing an identical rôle in the process of absorption, though this function was by no means as outspoken as in the capillary bed.

In addition to this use of perivascular channels as pathways of absorption, there occurred, under the influence of the intravenous saline, a diffuse penetration of the ependymal lining of the cerebral ventricle by the foreign solution. This appeared as a blue coloration in the gross, diminishing in intensity from the ventricular wall and of greater depth and amount in the fourth ventricle than in the third and lateral ventricles. This difference was probably to be explained by the greater dilution of the foreign solution in the lateral ventricles than in the fourth, due in part to the site of introduction of the foreign solution. Microscopic examination showed this ventricular coloration to be a diffuse penetration of the ependyma; precipitated granules

of Prussian blue were identified everywhere in the cytoplasm of these cells. Beneath the ependyma, the granules extended in diminishing numbers throughout the interfibrous network, to appear within the endothelial cells of the underlying capillaries, and occasionally to be found lying free within the lumina of these vessels. The mechanism here was apparently one of a diffuse absorption into the underlying capillary bed.

Histological study of the choroid plexuses of the fourth and lateral ventricles convincingly demonstrated that under these experimental conditions no absorption of the foreign solution occurred. Fairly dense blue precipitates covered all of the cellular tufts of the plexus but the cytoplasm of the columnar epithelial cells was entirely free from any of the Prussian blue particles. Occasionally the loose stroma of the plexus revealed scattered granules; in every case these could be traced outward to the subarachnoid space, which embryologically and structurally continues inward as the supporting tissue of the plexuses.

The gross and microscopic findings, then, indicated that the intravenous injection of a strongly hypertonic solution makes functional additional pathways of absorption for the cerebrospinal fluid through the perivascular system into the capillary bed of the nervous tissue and through the ependymal lining of the cerebral ventricles into the subependymal capillaries.

Granular suspensions. Similar experiments were carried out with the introduction of diluted India ink into the subarachnoid space at the time when the pressure of the cerebrospinal fluid was markedly reduced by the intravenous injection of a strongly hypertonic solution. The physiological effects of such an injection are well given in chart 4. This record differs from that of chart 3, largely in that the India ink caused but little alteration of either intracranial or systemic vascular pressures in comparison to the isotonic solution of potassium ferrocyanide and iron-ammonium citrate. In this experiment, the subarachnoid introduction of 4 cc. of the diluted ink over a period of 24 minutes caused the pressure of the cerebrospinal fluid to rise from minus 58 mm. to plus 5 mm.; cessation of the injection was followed by a drop in this pressure to minus 33 mm. The

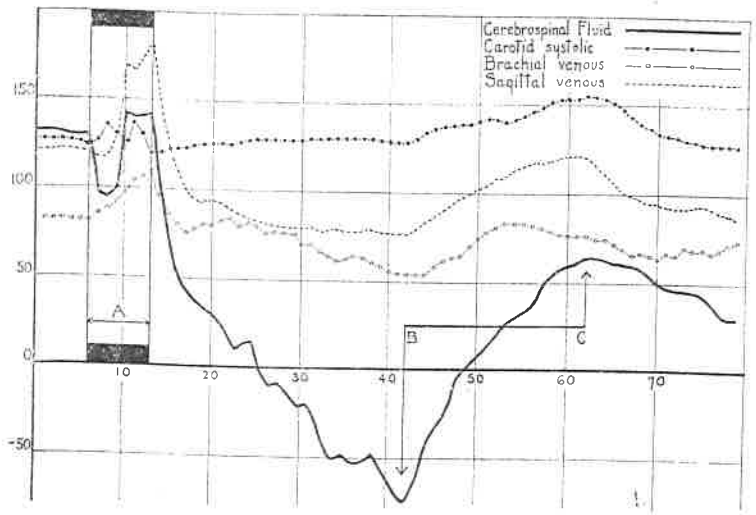

Chart 3 Experiment 34. Dog, weight 6550 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure); abscissae represent time in minutes. During blocked interval *A*, intravenous injection of 30 cc. of 30 per cent solution of sodium chloride. During interval from *B* to *C*, introduction of 4.1 cc. of isotonic solution of potassium ferrocyanide and iron-ammonium citrate into subarachnoid space.

alterations in vascular pressures during and after the period of introduction of the ink were of slight extent.

On gross examination, the nervous system of such an experimental animal showed a widespread distribution of the carbon particles throughout the subarachnoid space. The dura mater and the subdural space were both entirely free from the ink particles but the fourth, third and lateral ventricles contained small amounts of the black. On section of the nervous system, particularly in the upper cervical region and in the medulla, where the concentration of the carbon particles was maximal, a faint grayish haze seemed to extend inward from the subarachnoid space.

Microscopic study of the nervous systems and meninges of these animals was very interesting. The carbon particles were found uniformly scattered throughout the subarachnoid space,

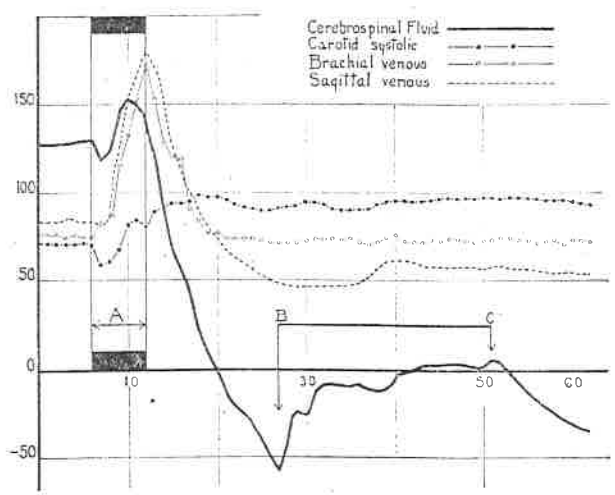

Chart 4 Experiment 17. Dog, weight 6160 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure); abscissae represent time in minutes. During blocked interval *A*, intravenous injection of 30 cc. of 30 per cent solution of sodium chloride. During interval from *B* to *C*, introduction of 4 cc. India ink, diluted 1 to 4, into subarachnoid space.

adhering to but not included within the cells lining this space. The core of each arachnoid villus was massed with the black but there was no evidence that any of the particles had passed through the mesothelial and endothelial cells into the lumen of the sinus. Within the cerebral ventricles the carbon particles were collected against the outer borders of the cells of the ependyma and of the choroid plexuses, but there was no indication of any absorption of the particulate matter by these elements.

The perivascular spaces of these animals, however, were partially filled with the carbon granules. These particles extended inward from the aggregations in the subarachnoid space for some distance (at a maximum, 3 mm.) but with the diminution of the caliber of the perivascular channel the granules were caught in the meshes and were not found in the pericapillary spaces. This indicates that the carbon particles were carried mechanically from the subarachnoid space into the perivascular

channels and that with the diminishing size of the space they became quickly filtered out of the fluid medium.

These observations may be interpretated as demonstrating that there was no free passage of particulate matter out of the subarachnoid space, even under the influence of the extraordinary osmotic pressure of the blood, occasioned by the intravenous injection of a strongly hypertonic solution.

DISCUSSION

The experimental findings presented in the foregoing pages have indicated that the intravenous injection of a strongly hypertonic solution effects a marked alteration in the process of absorption of the cerebrospinal fluid. Under conditions approaching the normal, with replacement of the spinal subarachnoid fluid and with control of all of the pressures concerned, absorption of the cerebrospinal fluid occurred through the arachnoid villi directly into the great dural sinuses; the foreign solution at no time entered the extensive perivascular system—a phenomenon strongly suggesting that normally the flow in these channels is from nerve-cell to subarachnoid space. But following the intravenous injection of the hypertonic solution, there occurred three processes of absorption of the foreign solution: the first of these was the normal mechanism of drainage into the venous system through the arachnoid villi; the second, a diffuse process of absorption through the ependymal lining of the cerebral ventricles into the subependymal vessels; and the third, a reversal of flow in the perivascular spaces, so that the capillary bed of the nervous tissue became capable of absorbing the fluid.

While in part the findings detailed in this paper have already been reported briefly, the present series of experiments permits the establishment of many of the previously accepted conceptions regarding the absorption of the cerebrospinal fluid on a firmer basis. For the first time, with the development of adequate methods for the determination of the cerebral venous pressures, it has been possible to correlate the pressures of the injection-

fluid with the cerebral venous pressure as well as with the existing pressure of the cerebrospinal fluid. In the replacement-type of injection (charts 1 and 2) the pressure of the foreign solution has been maintained at the same level above the cerebral venous pressure as existed in the animal before the replacement of cerebrospinal fluid in the spinal subarachnoid space was begun. And likewise in the experiments involving the intravenous injection of the strongly hypertonic solution, the supply of the foreign solution to the subarachnoid space has been accomplished without permitting the pressure of the injection-fluid to rise above a very low level or to exceed the pressure in the cerebral venous system (charts 3 and 4). The pathways taken by the foreign solutions under these conditions cannot therefore be interpreted as being due to abnormally high pressures of injection.

The findings here presented amply verify the previous conclusion (Quincke '72, Sicard et Cestan '04, Dandy and Blackfan '13, '14, Weed '14 b), that particulate matter does not leave the subarachnoid space in large amount, if at all. This holds true for the carbon particles when the replacement of the cerebrospinal fluid in the spinal subarachnoid space was accomplished with India ink; it is equally true when the ink was introduced into the subarachnoid space after the pressure of the cerebrospinal fluid had been markedly reduced by the intravenous injection of strongly hypertonic solutions. In both cases, however, the carbon granules were apparently carried almost as far in the subarachnoid space as was the suspending fluid: in the replacement experiments the granules reached the arachnoidal villi but did not pass through the cellular membrane while in the second type of experiment, the particles were carried for some distance inward along the perivascular channels. In neither case, however, was there evidence of absorption of the particles into the blood-vascular system.

With such a difference in absorption between granular suspensions and true solutions, it becomes necessary to ascertain the extent to which data derived from the use of a true solution of foreign salts may be relied upon as furnishing evidence of the

pathway of absorption. It is unfortunate that so far there is available only one solution which fulfills the rigid requirements for use in the subarachnoid space. In isotonic solution, the equal amounts of potassium ferrocyanide and iron-ammonium citrate are relatively non-toxic, are not attracted to any specific cellular elements and may be precipitated as insoluble granules which pass unchanged through the technical procedures for histological preparations. It is essential, however, that the fixation of the tissues be immediate so that the precipitation of the salts takes place before or simultaneously with the death of the cells; in dead tissue, the ferrocyanide-citrate solution diffuses readily and gives no index of the pathway of the fluid during life. The customary finding of the precipitated granules on the periphery of the leptomeningeal cells (where the concentration of the foreign salts is maximal) and of the freedom of the cytoplasm of these cells from the salts negatives any objections which may be advanced against the method as revealing only an ante-mortem or a post-mortem diffusion.

Accepting then the pathway of absorption as outlined by the injections of the true solutions under physiological conditions, it becomes evident that the present investigation supports the view advanced by the writer in 1914 that the normal pathway of absorption of the cerebrospinal fluid is in large part by way of the arachnoid villi into the great dural sinuses. The experimental verification of the conception is however now placed on firmer grounds by more adequate control of the physiological conditions. Mott's theory of the absorption of cerebrospinal fluid by way of perivascular channels from the subarachnoid space into the cerebral blood vessels is not supported by the results of these replacement-experiments, nor was the conception upheld by the former experiments of the writer. Likewise, the hypothesis of a diffuse absorption of the fluid by the blood vessels of the pia-arachnoid, advanced by Dandy and Blackfan, has not been verified by either the earlier or by the present investigation of the writer. For, with the leptomeningeal vessels all clothed by the mesothelial cells lining the subarachnoid space, it is necessary for the fluid to pass through these

cells to reach the vessels; no evidence of this passage has ever been obtained, since the mesothelial cells function as efficient fluid retainers. Other arguments against the hypothesis of Dandy and Blackfan have already been presented elsewhere (Weed '14 b, '17, '22).

It is interesting that under the influence of an increased concentration of salt within the blood stream, Mott's ('10) theory of drainage of the cerebrospinal fluid becomes established. This reversal of flow in the perivascular channels from subarachnoid space to nerve-cell and capillary bed of the nervous system was first briefly pointed out by Weed and McKibben in 1919; it has since been substantiated by Foley ('21) who made use of the same methods of investigation. In the present study, with adequate physiological controls, the use of these potential channels as actual pathways for the absorption of fluid becomes amply demonstrated. These channels had previously been injected to their ultimate terminations about the nerve cells by the use of very high pressures (ca. 150 mm. Hg) in the subarachnoid space and also by the maintenance of the normal subarachnoid pressure simultaneously with the production of a cerebral anemia. Under normal conditions, however, the flow in these perivascular channels is apparently from nerve-cell to subarachnoid space, the small amount of fluid poured out through these channels being added to the cerebrospinal fluid in the subarachnoid space.

The increase in the salt-content of the blood, in addition to the reversal of the flow in the perivascular channels, opens up an additional pathway of absorption through the ependymal lining of the cerebral ventricles. Normally, if one may judge from the results of the replacement-experiments, there is no absorption of fluid from the ventricles either by the ependymal cells or by the choroid plexuses; under the influence of the intravenous injection of a strongly hypertonic solution, a diffuse absorption through the ependymal lining occurs. This observation finds support in the work of Wislocki and Putnam ('21) who showed that in the dilated ventricles of a hydrocephalic animal absorption occurred in small amount through the ependy-

mal cells but not through the epithelial cells of the choroid plexuses. Likewise, Nañagas ('21) demonstrated an increased rate of absorption from similarly dilated cerebral ventricles following the intravenous injection of strongly hypertonic solutions; the pathway of absorption was solely through the ependyma and not through the more highly differentiated cells of the choroid plexuses. Foley ('21), however, reported that ventricular absorption, under the influence of hypertonic solutions, was through the epithelial cells of the choroid plexuses. The present series of experiments, carried out with adequate physiological controls, is convincing in demonstrating that the ventricular absorption, induced by the intravenous injection of the hypertonic solution, is through the ependymal cells and not through the epithelial covering of the choroid plexuses.

The differences in the processes of absorption of the cerebrospinal fluid under normal conditions and under the influence of the intravenous injections of strongly hypertonic solutions offer an exceptional opportunity for speculation regarding the physical mechanisms involved. Under normal conditions, the pressure of the cerebrospinal fluid is almost always in excess of the cerebral venous pressure as determined in the superior sagittal sinus (Weed and Hughson '21 b). Explained on the simplest basis, then, the normal process of absorption may well be merely a filtration of a true solution from a point of higher pressure (subarachnoid space) to a point of lower pressure (dural venous sinus) through the cellular membranes of the arachnoid villi. But in the final passage of this fluid through the membrane, osmosis and diffusion may be factors of importance, though the evidence supporting their inclusion in the process is incomplete. When an intravenous injection of a strongly hypertonic solution is administered, the osmotic pull of the blood becomes extreme and water is attracted into the blood stream from all available reservoirs. The cerebrospinal fluid as well as the intrinsic fluids of the brain (Weed and McKibben '19 a, Weed and Hughson '21 a) contribute to this supply of water demanded by the increased salt-content of the blood; the process of absorption of the cerebrospinal fluid becomes immediately one controlled

largely by the extraordinary osmotic pressure of the blood. This is shown by the opening up of all anatomically available channels for fluid passage; the absorption of the foreign true solutions takes place from the perivascular channels into the capillary bed for the nervous system and through the ependynal cells of the ventricles into the subependymal capillaries, as well as through the normal pathways of absorption in the arachnoid villi. The increase in the osmotic pressure of the blood, under these experimental conditions, accounts, however, only for the absorption of water from the foreign solution; the additional factor of diffusion must be assumed to function in the passage of the foreign salts in solution through these membranes. These two factors, osmosis and diffusion, must also act in the membranes covering the arachnoid villi, for under the experimental conditions established, the factor of filtration from the point of higher pressure to a point of lower pressure becomes inoperable; the subarachnoid pressure here is below that in the superior sagittal sinus. But the villi are covered with a cellular membrane possessing certain qualities of permeability; and under the heightened osmotic pull of the blood, osmosis and diffusion apparently become operable at the membrane, so that absorption takes place. It is apparently only where extreme cellular differentiation has proceeded (as in the epithelium of the choroid plexuses for fluid-secretion and in the mesothelium of the leptomeninges for fluid-retention) that these processes of osmosis and diffusion cannot operate even with the extraordinary increase in salt content of the blood. The other potential anatomical channels are all opened up as pathways for the absorption of the cerebrospinal fluid. The interpretation of these findings on the basis of the extreme importance of osmosis and diffusion in the process of absorption of the cerebrospinal fluid, under the abnormal condition of an exceptional osmotic pressure within the blood stream, suggests that the importance of these factors in the normal process of absorption may have been minimized.

CONCLUSIONS

The pathway of absorption of the cerebrospinal fluid into the blood stream under normal conditions is by way of arachnoid villi into the great dural venous sinuses. Under the influence of an increased salt-content of the blood, effected by the intravenous injection of strongly hypertonic solutions, absorption takes place also by way of the perivascular channels and through the ependymal lining of the cerebral ventricles into the capillary bed of the nervous system. In the normal process, filtration may be the physical factor of greatest importance, but after the intravenous injection of strongly hypertonic solutions, osmosis and diffusion apparently play the only active rôles.

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