THE CEREBRO-SPINAL FLUID. IV[1]. CIRCULATION.

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CONTENTS.

It has been shown both in men and animals that cerebro-spinal fluid is being continually formed. When it is withdrawn it is soon replaced; after cranial injuries large quantities of the fluid may flow away regularly and constantly; in certain pathological conditions in which the escape of the fluid is prevented, symptoms of cerebral compression occur. These facts are well recognised and require no comment(1). Artificial hydrocephalus in animals also can be produced by plugging the lower end of the aqueduct of Sylvius(2).

The fluid formed in the cerebral ventricles by the choroid gland has free communication with the fourth ventricle, with the subarachnoid space by the foramen of Magendie and the foramina of Luschka, and fills up all the spaces around the brain and spinal cord. The quantity contained in the ventricles of the brain, the central canal of the cord, and the subarachnoid space has been estimated by several observers and in man averages 100–130 c.c.

Cerebro-spinal fluid fills the peri-vascular and peri-neuronal spaces, and thus comes into contact with the tissue elements, and this fact

[1] Parts I, II and III have been published in this *Journal*, XLVII, p. 215, 1913, XLVIII, p. 128, 1914, and XLVIII, p. 317, 1914. A preliminary account of the present investigations was published in the *Proc. of the Physiol. Soc.*, March, 1912, p. vii, this *Journ.* XLIV.

has given rise to the view that the fluid serves as the "lymph of the brain." Weed(3) has emphasised this opinion; he believes, however, that the main source of the fluid is from the choroid gland, and that the fluid drains into the meningeal vessels. Several observers have noted that the absorption or disappearance of fluid occurs much more rapidly in the region about the base of the brain than in other parts, such as the spinal district, a fact which is easily confirmed. Other investigators maintain the view that the fluid escapes along the lymphatics of the cranial and spinal nerve sheaths and passing through the paravertebral lymphatic glands eventually arrives at the thoracic duct and thus the venous circulation. The general method adopted in investigations of this nature is to ascertain how coloured or other easily recognisable substances pass either from the cranio-spinal cavity to the blood or lymph-vascular systems, or in the reverse direction from the blood to the cerebro-spinal fluid. Some of our investigations have been carried out by such methods and the results strongly support the view that the fluid normally passes into the blood and especially into the large venous sinuses; they do not absolutely exclude the possibility that the fluid may in part pass into the cerebral capillaries and veins.

Flatau(4) made numerous injections into the subarachnoid space of rabbits, and concluded that the fluid follows the course of the sheaths of nerves, especially of the olfactory nerves, then passes into the lymphatic network of the nasal mucous membrane and arrives at the glands of the neck. Sicard(5) injected Chinese ink and found that the subarachnoid lymphatics became impregnated with it; the experiments were performed in dogs, but as the examination of the tissues was postponed for some months after the injection was made, the results are not of much value. Cathelin(6) injected easily recognisable salts and concluded that absorption is *via* lymphatics which enter the thoracic duct. Hill(7), on the other hand, states that methylene blue added to the cerebro-spinal fluid passes straight into the venous sinuses, and in 10–20 minutes is excreted into the gastric juice and urine. The lymphatics in the neck are not coloured in this time, and even after an hour's steady injection the deep cervical lymphatic glands are found only partly tinged with the blue colour.

Adamkiewicz first showed that there is a free communication between the subarachnoid space and the longitudinal sinus. Cushing(8) agrees that this is so, but questions the truth of Key and Retzius' hypothesis(9) that the Pacchionian bodies act as a filter since these

structures are absent in the infant and in lower animals. Cushing was at this time led to believe that there are patent valved orifices leading into this venous channel, which allow the fluid to pass in, but do not allow the blood to flow back. More recently he has abandoned this view in favour of that of his colleague Weed(10) who regards the microscopic arachnoid villi as the situations where the fluid filters through into the meningeal sinuses. Frazier and Peet(2) introduced methylene blue and trypan blue into the subarachnoid space: the latter pigment which was more quickly absorbed took twelve hours to reach the cervical chain of lymphatic glands, but phenolsulphonaphthalein could be detected in the torcular blood within two or three minutes and in the urine in five and a half minutes. They calculate that 50–60 % of the normal fluid is absorbed within two hours, but regard absorption by lymphatics as an almost negligible factor.

The quantity of fluid which can be run into the cranio-vertebral cavity in a short space of time shows that the exit is an easy one. Thus Duret(11) saw 583 c.c. of water pass in, in the course of two hours. Naunyn and Schreiber(12) injected 400 c.c. of saline solution in 105 minutes at the pressure of 100 mm. of mercury. Falkenheim and Naunyn(13), using pressures varying from 200 to 800 mm. of water, record rates of flow into the cerebro-spinal space varying from 0·2 to 6·5 c.c. per minute, variations in the arterial pressure having little or no influence on the absorption. On the other hand the passage of substances in solution from the blood into the cerebro-spinal fluid is extremely difficult. A. and E. Cavazzani(14) injected potassium ferrocyanide into the peritoneum of dogs and rabbits, and found no trace of it in the cerebro-spinal fluid an hour later, and potassium iodide gave similar results; negative results were also obtained by Behring, Jacobs and others(15). Recent literature on the use of salvarsan in the treatment of syphilis has shown that this drug given in the usual way has no effect on the syphilis parasite when the latter is lodged in the annexes of the cerebro-spinal space as in locomotor ataxy, and general paralysis. It has further been shown that drugs which will not pass from the blood to the cerebro-spinal fluid will produce marked results when introduced directly into that fluid. Thus Camus(16) found that barium chloride which is a very active poison to the central nervous system will kill a rabbit of 2 kilogrammes weight when one-tenth of a milligramme is introduced into the subarachnoid space, whereas the lethal dose when the salt is given subcutaneously is one thousand times greater. The use of salvarsan

in locomotor ataxy and similar post-syphilitic affections *via* the cerebro-spinal fluid has been abandoned as it is fatal not only to the syphilitic organism, but also to the patient(17).

Judging by previous literature alone, the balance of evidence shows that there is an intimate communication between the cerebro-spinal fluid and the blood stream rather than with the lymph-vascular system.

It is generally admitted that the perivascular and perineuronal spaces communicate with the subarachnoid space and are filled with cerebro-spinal fluid; the powerful action of drugs which affect nerve cells when introduced into the subarachnoid space is thus clearly intelligible.

Authors who have investigated the anatomy of the vascular arrangements in the central nervous system differ as to whether or not a true lymphatic system exists in addition to the system of intracerebral and intraspinal spaces above alluded to and which may be regarded as prolongations of the subarachnoid space. Weed, for example, maintains that the only representative of the lymphatic system consists of these perivascular and perineuronal spaces and that these are filled with cerebro-spinal fluid. Cathelin describes a system of true lymph vessels in addition. There is, of course, no question that the peripheral nerves have true lymph vessels.

1. *Absorption from the cerebro-spinal fluid.*

It has been noted in our third paper (18) that when the cerebro-spinal pressure was raised by pumping saline solution into the subcerebellar cisterna, the pump was required to be kept working to maintain the pressure at a constant level. This means that the salt solution leaves the cranio-spinal space rapidly. This absorption of saline is readily shown by connecting the cannula from the cisterna to a burette filled with physiological saline solution, then by periodically filling the burette the rate of inflow can be noted. Thus in one dog 200 c.c. of saline solution flowed in in half an hour under a pressure of 500 mm. of water: this figure is considerably in excess of that which usually obtained though in all instances the rate of absorption was considerable.

In order to determine how absorption is produced, small quantities of crystalline substances were dissolved in 5 c.c. of saline solution and injected into the cisterna in the usual way; the first substances chosen were those easily recognisable by their physiological action, such as atropine, adrenaline and nicotine. With these drugs the latent period before their effect is obvious is surprisingly short, though animals vary to some extent. Sometimes the onset of action is so rapid as to simulate an intravenous injection. Thus the injection of 1 or 2 c.c. of a ·1 %

solution of atropine sulphate produced paralysis of the vagi in a cat, in less than four minutes, whilst the same dose in a dog washed in with a little saline solution induced vagal paralysis in about one minute. With adrenaline the effect is still more striking though the rapidity of action varies a good deal in different animals. Usually after an injection into the subcerebellar cisterna a short latent period ensues of from 10 to 30 seconds, then cardiac acceleration becomes obvious (the vagi having been cut) and the blood-pressure rises. Fig. 1 shows

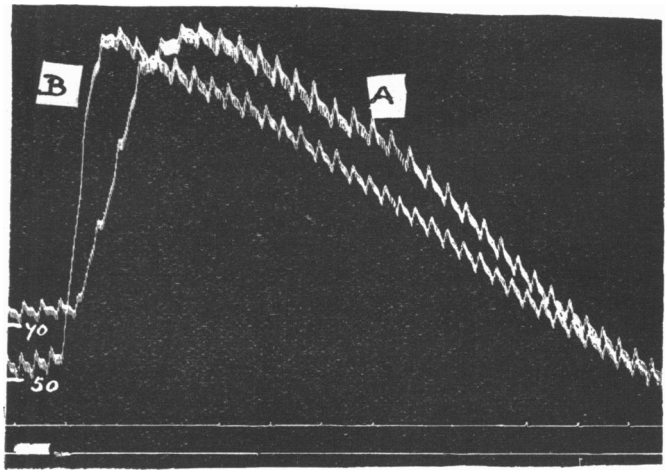

Fig. 1. Dog. Tracing of arterial pressure (femoral artery). At the indicated mark 2 c.c. of ·01 % adrenaline was injected into the subcerebellar cisterna and the curve is shown in A. The drum was then turned back and the same dose was injected into the femoral vein (B). Time = 5 secs.

that this rise may be almost as vertical as that which obtains after an intravenous injection but occasionally the rate of rise is slower and resembles a slow intravenous injection of a dilute solution. For purposes of comparison in this figure a record of intravenous injection is superimposed, the time and rate of injection in each case being approximately the same. In all these injections the cannula was opened before the drug was given and 2 or 3 c.c. of cerebro-spinal fluid allowed to escape, so as to prevent, as far as possible, any undue increase in cerebro-spinal pressure. Further, in all cases the cerebro-spinal fluid was completely free from all traces of blood, so that there could have been no communication with blood vessels injured during the puncture.

Nicotine in doses of 1 or 2 mgms. acted like adrenaline on the vascular system and was readily absorbed from the cerebro-spinal fluid into the blood (Fig. 2).

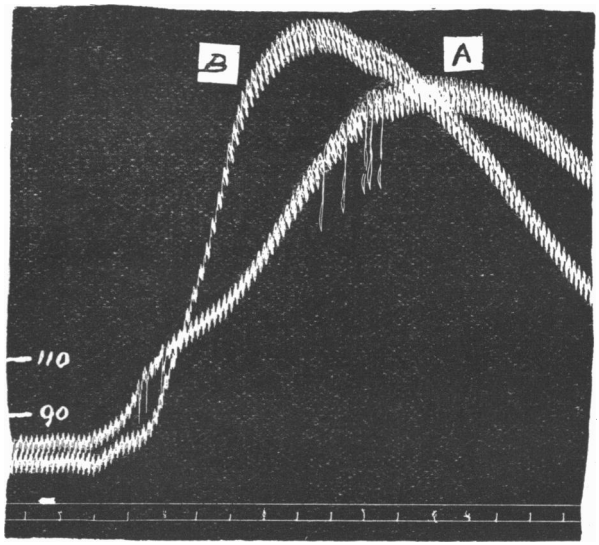

Fig. 2. Dog. Arterial pressure. Shows the effect of two injections of nicotine. The first, curve A (3 c.c. of a ·1 % solution), was into the subcerebellar cisterna, and the second (2 c.c.), curve B, into the femoral vein: superposed as in Fig. 1. Time = 5 secs.

If the animal was deeply anæsthetised with urethane no effect directly on the motor cells of the cord was obvious. If, however, the animal was anæsthetised with A.C.E. mixture, marked twitchings and irregular contractions occurred in the muscles of the head, forelimbs and neck. Such twitchings by the direct application of nicotine to the cord have already been recorded by Langley for the skate and Dixon for the frog. Their particular significance here is that they never extend to the lower part of the body and we shall adduce further evidence to show that solutions injected into the cerebellar cisterna do not move downwards towards the cord.

One other example of an easily detectable crystalline substance may be mentioned: this is β-iminazoylethylamine, which lowers blood-pressure. Injections of this substance produced effects which followed the same general law; when injected into the subcerebellar cisterna the fall of blood-pressure ensued after a slightly longer latent period

and was not quite so profound as that caused by an equal dose injected into the vein (Fig. 3).

These experiments show clearly that crystalline chemical substances are rapidly absorbed from the cerebro-spinal fluid into the blood.

Since the publication of our preliminary note, Auer and Meltzer (19) have made some intraspinal injections of adrenaline which they expected would have no stronger action on blood-pressure than that of a subcutaneous injection. They employed monkeys and injected large doses; the arterial pressure rose slowly and reached its maximum in a few minutes and remained high for a considerable time. The injections were made into the lumbar region. We have made some injections into the lumbar region, but there, as we have already stated, absorption is comparatively slow and several minutes are required before any noticeable effect is observed.

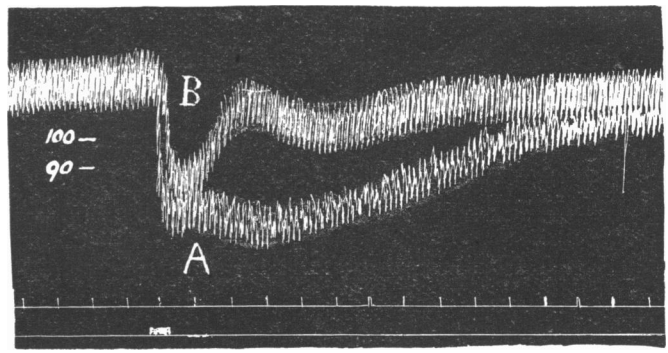

Fig. 3. Dog. Arterial pressure. Shows the effect of injecting β-iminazoylethylamine (5 c.c. of a ·05 % solution) first into the cerebro-spinal fluid (B), and second into the femoral vein (A). The two curves are superposed as before. Time = 5 secs.

Some experiments were made with sodium salicylate. 10 c.c. of a 5 % solution of sodium salicylate were introduced into the subcerebellar cisterna of a dog, and the blood was collected in samples at periods varying from one minute to fifteen minutes later: namely at the end of the first, fifth, tenth, and fifteenth minutes. Each sample consisted of 10 c.c., and the samples were collected simultaneously from the torcular and the femoral artery. The injection produced no obvious effect on the animal. The first sample, namely that collected at the end of the first minute, contained no salicylate or at any rate an amount insufficient to be detected. The sample collected next (at the end of the fifth minute) gave the salicylate reaction, and this was more intense in the venous than in the arterial sample. The two later samples gave the reaction still better, but the difference between the venous and

arterial blood had now disappeared, or if anything the reaction was rather more intense in the arterial blood. The early appearance of the drug in the torcular blood is another item in the proof that the cerebro-spinal fluid passes readily into the blood vessels of that region.

The method of analysis of the blood was as follows: the 10 c.c. of blood were diluted with 20 c.c. of distilled water and a little hydrochloric acid was added. The mixture was left on the boiling water bath for ten minutes and then filtered. The filtrate was shaken out with petroleum ether in a separating funnel three times, a few drops of alcohol being added to clear up any emulsion. The extract was evaporated nearly to dryness on an electric heater; the residue was taken up with a small measured volume of water, and two drops of ferric chloride solution added. The intensity of the violet coloration so produced was taken as a rough index of the relative quantities of the salicylate in the different samples of blood.

Other experiments with potassium iodide and potassium ferrocyanide gave similar results and no special description is necessary.

So far then it is quite clear that crystalline substances of a relatively simple chemical constitution find their way from the cerebro-spinal fluid to the blood with extraordinary rapidity.

It has been stated that pituitary secretion finds its way into the cerebro-spinal fluid and normal cerebro-spinal fluid always contains a trace of some substance which has all the characteristic actions of pituitary extract. Pituitary extract then should exert its ordinary systemic actions when injected into the cerebro-spinal fluid, and this we find to be the case. 2 c.c. pituitary extract (Burroughs, Wellcome and Co.) injected into the subcerebellar cisterna and washed in with saline causes a slow and gradual rise in blood-pressure, reaching a maximum several minutes after injection. If, when the rise is at a maximum or before this time, the contents of the cerebro-spinal spaces are emptied, that is the pressure is removed by means of the syringe attached to the cannula in connexion with the space, the blood-pressure commences to fall almost at once. In one experiment on a lactating cat the injection of pituitary in this fashion caused a free secretion of milk. It cannot be doubted then that pituitary is readily absorbed in this manner. The comparatively rapid action observed indicates that "pituitrin" is readily diffusible and so its molecule cannot be a very large one.

Secretin was prepared fresh from the alimentary canal of the dog or cat. It was employed because it has been suggested that its molecule is a large one and that its solution is possibly colloidal in nature. If it is non-diffusible then it would hardly be expected that absorption

would occur from the cerebro-spinal fluid sufficiently rapidly to produce much flow of pancreatic juice. The experiments were performed in the usual way with the exception that a cannula was placed first in the pancreatic duct. A small injection of secretin was first made into the femoral vein and in each of three experiments this produced the typical secretion after a latent period of about two minutes. After a suitable interval a larger dose of secretin was injected into the cerebro-spinal fluid, no effect on pancreatic secretion was however obtained for four or five minutes and then the secretion was very small, only about six drops being secreted. In the same animals control injections of adrenaline showed that this substance was readily absorbed into the blood stream from the cerebro-spinal fluid. Secretin then though absorbed is clearly not absorbed at the same rate as adrenaline and other drugs with a relatively small molecule; nevertheless it is absorbed much more rapidly than proteins.

As an example of a protein Witte's peptone was chosen as it produces readily recognisable physiological effects. We used this non-diffusible substance as a means of settling the question whether the transference of materials from the cranio-vertebral space to the blood takes place through orifices or by diffusion into the blood vessels. We find that colloidal materials of this kind do not produce their specific effects if they are introduced into the subcerebellar cisterna; that is they enter the blood if at all with extreme difficulty, and therefore not through valvular orifices.

A 10 % solution of Witte's peptone was employed; 10 to 20 c.c. of this (a dose sufficient to produce marked effect when injected into the blood stream) was injected into the subcerebellar cisterna of small dogs. No fall of blood-pressure occurred and no change in the coagulability of the blood was noted within the next hour or more. A transitory rise of arterial blood-pressure occurred but this was manifestly the result of the increased pressure of so much extra fluid in the medullary region, and was produced equally well by the same amount of a neutral physiological saline solution. The blood-pressure soon returned to its normal level. The injection of the same or even half the amount of the peptone solution into the femoral vein produced the well-known and permanent drop of arterial pressure, and the coagulability of the blood was lost either wholly or completely within a minute or two.

A tracing is appended to illustrate these points (Fig. 4). The following protocol of an experiment which is quite typical of the others sufficiently explains the effect.

May 21, 1912. Dog. Urethane and morphine; artificial respiration. Tracing of femoral blood-pressure taken. Cannulæ inserted into the subcerebellar cisterna, the femoral vein, and the opposite femoral artery. Injection of 10 c.c. of a 10 % solution of Witte's peptone into the subcerebellar cisterna induced a slight rise of arterial pressure showing Traube-Hering waves due to increased pressure on the medullary centres, caused by the injection. These did not occur in the experiment from which Fig. 4 was taken. The blood-pressure soon returned to normal and remained there for the succeeding 30 minutes. At the end of this time the same dose of the peptone solution was injected into the femoral vein which caused an almost immediate fall of pressure which persisted until the animal was killed.

Coagulability of blood from femoral artery.

11.23. Before injection. Blood sample clotted in five minutes.
11.39. Injection of peptone into subcerebellar cisterna.
11.40. Sample of blood clotted in 5½ minutes.
11.45. „ „ 4 „
11.57. „ „ 5 „
11.58. Injection of peptone into vein.
11.59. Sample of blood clotted in 24 minutes.
12.3. Sample of blood did not clot while it was watched for the succeeding two hours

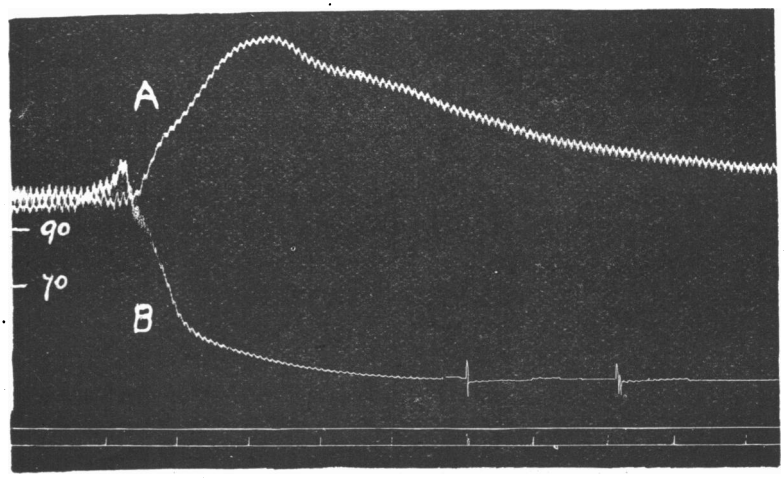

Fig. 4. Dog. Arterial pressure. Shows the effect of injecting 5 c.c. of a 20 % solution Witte's peptone into the cerebro-spinal fluid (tracing A) and into the femoral vein (tracing B). The two curves are superposed as in previous figures. Time = 10 secs.

In this animal a preliminary control experiment was performed with nicotine, and the result has already been given in Fig. 2. This shows that an easily diffusible substance passed readily into the blood stream from the cerebro-spinal space.

One further experiment was performed on a dog which had received an injection of horse serum 23 days previously. This animal was

anæsthetised with A.C.E. mixture only. It was found that when a small injection of the same serum was administered into the cerebro-spinal fluid no result was obtained, but that the same injection into a vein produced typical signs of anaphylaxis with distressed respiration and a profound fall in arterial pressure.

These facts then show that crystalline substances find their way very readily into the vessels and produce their typical specific effects, whilst substances possessing a large molecule like those of a protein nature are absorbed very slightly or not at all.

2. *Absorption of substances from the general circulation into the cerebro-spinal fluid.*

The experiments we have made under this heading are few, and they verify the results of previous workers. All observations show that substances foreign to the cerebro-spinal fluid obtain access to it only with difficulty, and we believe are never present in more than traces. A few drugs do get through; thus Crowe (20) showed that hexamethylene tetramine (urotropine) administered internally can be detected in the cerebro-spinal fluid and it is for this reason that it is employed in some forms of meningitis. This observation has been confirmed by Hald (21), who also notes the passage of other substances such as alcohol, chloroform and acetone.

We performed a few experiments with crystalline substances which show in each instance that after injection into the blood the merest traces of them obtain access to the cerebro-spinal fluid and only after a long interval. The description of one experiment will suffice to make this clear.

Goat. Ether and urethane. At 1.20 p.m. injected into the femoral vein 50 c.c. of a 6 % solution of potassium iodide and 50 c.c. of a 5 % solution of sodium salicylate. The cerebro-spinal fluid was collected at certain periods from the subcerebellar cisterna.

Time	Amount of cerebro-spinal fluid drawn off	Remarks
1.40 p.m.	6 c.c.	No drugs detected.
2.0	5 c.c.	„ „
2.20	4 c.c.	0·001 % salicylate. Trace of iodide.
2.40	3 c.c.	Amount of both slightly increased.
3.0	3 c.c.	Amount of drugs about the same.
4.0	3 c.c.	Traces of drugs only.
6.0	3 c.c.	No drugs detected.

The blood-pressure remained high throughout, about 135 mm. Hg.

Similar experiments have also been made with urotropine, and it is quite easy to show that small amounts of this substance find their

way into the cerebro-spinal fluid. We have, however, completely failed to detect there even traces of formaldehyde, to which the antiseptic effect of urotropine has been attributed.

The choroidal epithelium which secretes the cerebro-spinal fluid forms an effective barrier which prevents the access into it of foreign materials, and thus the nerve-cells which this fluid bathes in the perineuronal spaces are protected from the action of metallic and other poisons (toxins) to a very considerable degree.

3. *The injection of coloured fluids.*

In order to trace the pathway of absorption one other method was adopted, viz. the injection of coloured fluid into the cerebro-spinal fluid. Our experiments were made in 1911 and our preliminary account of them was published more than two years before Weed's paper(22) appeared. Weed gives a useful historical account of our knowledge of the cerebro-spinal fluid.

We have employed as stains methylene blue; carmine; potassium ferrocyanide, and iron ammonium citrate, the tissues being subsequently treated with hydrochloric acid as recommended by Weed. In a few experiments we used a granular injection, Indian ink. The chief difference in the methods that we adopted in this type of experiment from those of previous observers consists in the fact that the injections were made without any form of cutting operation, and they were made during life on animals which were anæsthetised and kept alive for from one to four hours after the injection started; the injection was made high up, namely, into the subcerebellar cisterna. The animals were killed either by bleeding, chloroform or air embolus and examinations were made of the fresh tissues soon after death. Just before the animal was killed the cannula was opened so that all excess of fluid could drain out. Immediately after the cannula had been inserted the clear cerebro-spinal fluid was allowed to drain out for about five minutes, then the fluid for injection contained in a flask was connected by rubber tubing with the cannula, air being carefully excluded. The coloured fluid was now allowed to run in whilst the animal was breathing normally, and this process was continued for half to one hour. It is necessary that the solution should approximate in osmotic pressure to that of normal saline and it must be at body temperature. The pressure was varied in different experiments: the lowest pressure employed was 15 cm. of water, and the highest 50 cm.

In some of the experiments a cannula was placed in the torcular so that the blood from the sinuses could be tapped periodically. Methylene blue could be detected in the torcular blood within a few minutes, at a time when it was quite impossible to detect it in ordinary arterial blood. This agrees with the observations we have described already with drugs, and leaves no doubt that the fluid is absorbed directly into the venous sinuses.

Post-mortem examination consisted in the thorough exposure of the brain and spinal cord; when methylene blue is employed a sufficient time must be given for oxidation to occur. Everywhere under the dura mater on the dorsal surface of the brain and cord the colouring matter is present and causes deep staining. The fluid does not however find any exit along the sheaths of the spinal nerves. The portions of these nerves within the dura mater are naturally deeply stained but beyond this there is a sharp line of demarcation, and the nerves are absolutely white outside the spinal canal.

We were unable to trace any blue colour along the lymphatics either in the cranial or spinal region. There is no general staining of the lymph glands; a few patches of blue can sometimes be seen in one or two of the lymphatic glands in the neck region, and these might be explained quite easily by the dye having reached them *via* the blood; but in most cases such patches are completely absent.

In two dogs we inserted a cannula into the thoracic duct before injecting methylene blue; the lymph was collected and freely exposed to the air but even after half an hour not a trace of blue was to be seen in the issuing lymph, and in the post-mortem examination half an hour later the lymph was still clear. Atropine and adrenaline were injected before death and these readily left the cranio-spinal cavity and produced their usual effects on the vagus and blood-pressure respectively. The methylene blue was nevertheless absorbed, as a trace was detected in the urine. In only one cat which was examined post-mortem after a similar injection of methylene blue, the lymph in the thoracic duct had a faint greenish tinge, but we do not attach much importance to this isolated observation, as the autopsy was performed after a long injection period, and the blue pigment might therefore quite easily have reached the thoracic lymph through the inter-mediation of the blood.

The blood vessels and sinuses especially in the meninges and bones of the skull, and of the brain itself and in the vertebral column, are on the other hand filled with deep blue blood; but the amount of blue

staining diminishes towards the posterior part of the spinal cord, and even after a period of an hour or more of injection the lower portions of the cord and vertebral column are completely free from the blue stain.

The pigment passes into the central canal of the cord after an hour's injection to about the mid dorsal region and into the ventricles of the brain. In most of the experiments the animal was on its side and in these cases the lateral ventricle which was uppermost received very little and sometimes no colouring matter, whilst the lower was invariably stained, especially the choroid plexus, and the colour was easily traceable to the aqueduct of Sylvius. The colouring fluid passes into all the fissures of the cerebrum and cerebellum.

It is evident from this, as well as from injections made directly into the lumbar region, that the movement of the cerebro-spinal fluid and the possibilities of its absorption are considerably less in the lower spinal region than they are in the lower regions of the brain and upper part of the spinal cord.

These experiments support the view that the main communication of the fluid is with blood especially in the region of the cranial sinuses, and the lymphatics are not the channels of absorption. We have found the same with other substances; absorption is always much slower in the lower spinal districts. This is tacitly recognised by those who produce spinal anæsthesia by injecting drugs of the cocaine group into the cerebro-spinal fluid. The horizontal position in such cases is regarded as dangerous, for if the anæsthetic reaches the bulbar region, it is rapidly absorbed and produces general and serious results.

In two or three animals immediately after death we tried whether methylene blue forcibly injected into the blood stream appears in the cerebro-spinal fluid; not a trace of blue was ever seen in that fluid; the same is true for air forcibly blown into the veins; no bubbles issue from the blood into the cerebro-spinal fluid.

Apart from the sinus blood which allows only substances in solution to pass into it, evidence was obtained that the cerebro-spinal fluid passes out in other ways though probably in normal conditions only to a trifling extent. The nose is certainly the most important of these: the cribriform plate takes on an intense stain and the sheaths of the olfactory nerves are deeply coloured, the ethmoidal cells and turbinated mucous membrane are also blue (see Fig. 5). Some communication then clearly exists between the nose and the cerebro-spinal fluid. This in view of epidemic cerebro-spinal fever is of some importance. In our third paper(18) it was pointed out that one symptom of increasing

greatly the cerebro-spinal pressure is salivation and running from the nose. When the pressure has been raised greatly (200 or 300 mm. of mercury) by means of the methylene blue solution we have failed to observe that either of these secretions contains any blue colour. This negative observation is opposed to the observation of Weed who,

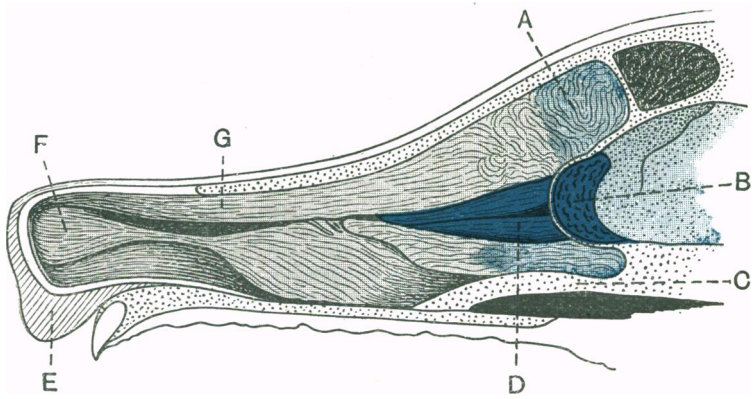

Fig. 5. Section through dog's nose. Taken a little inwards from the median line. *A*, cells; *B*, cribriform plate; *C*, vomer; *D*, ethmoidal cells; *E*, upper lip; *F*, concha inferior; *G*, concha superior.

This picture represents the appearances some three hours after death. During life the animal for a period of an hour had the subcerebellar cisterna connected with a reservoir containing a solution of methylene blue at a pressure of 30 cms. of water. Before the animal was killed time was allowed for the escape of all excess of fluid.

using ferrocyanide as an easily detectable substance, found it in the fluid which drips from the nose, suggesting the possibility of a cerebro-spinal rhinorrhœa. The mouth, pharynx and tongue are not usually coloured.

Evidence of a like nature also exists and has been described in detail by other workers to show that some fluid finds its way out along the sheath of other cranial nerves. The optic nerve is certainly the most important of these. It is known that the arachnoid membrane extends for short distances along the cranial nerves and this appears to be connected with the perineural space and it is this which is injected. In the case of the optic nerve the colouring matter passed out along the nerve sheath and over the posterior surface of the eyeball by way of the optic subarachnoid into Tenon's capsule.

The vagus nerve takes on a blue colour as far as the ganglion of the trunk. The spinal accessory is also stained till it has passed through the foramen in the skull. The nerves in the middle ear are stained, so also are the sacculus and utriculus.

4. *Pituitary extract.*

In an earlier communication (24) we stated that pituitary extract administered intravenously had no decided influence on the rate of secretion of cerebro-spinal fluid. Since then Weed and Cushing (23) have stated that extracts of the posterior lobe of the hypophysis cause a very marked increase in secretion. The question is important seeing that the pituitary secretion passes into the cerebro-spinal fluid and will therefore bathe the choroid plexuses.

It may be stated at the outset that both statements are correct for the conditions under which the experiments were performed, and it is therefore necessary for us to make clear how two such divergent statements can be reconciled. In our first paper (24) we laid great stress on the fact that deprivation of oxygen, partial asphyxia, however produced, is a powerful stimulus to the secretion of cerebro-spinal fluid. To avoid all such errors we anæsthetised the experimental animals deeply with urethane and morphine and performed a forceful artificial respiration, and we found it was only by this means that we were able to exclude the respiratory factor. Such drugs as barium, β-iminazoylethylamine and numerous others injected intravenously, which under the conditions stated produce no effect on cerebro-spinal secretion, nevertheless when injected into animals which are breathing naturally and are anæsthetised with some volatile anæsthetic produce a great increase in the rate of cerebro-spinal secretion.

Weed and Cushing used animals with natural respiration and which were kept unconscious by the employment of intra-tracheal insufflation with an unchanging tension of volatile anæsthetic. They state that after an injection of pituitary extract a considerable increase of the cerebro-spinal secretion occurs and that coincident with this period a slight amplitude of the respiratory stroke is registered. They also draw attention to the fact that we state that an increase in the rate or amplitude of respiration produces a decrease in the flow from the cannula and draw the conclusion that respiration cannot be responsible for the increase of the secretion. The position then we have to determine is how far the results of Weed and Cushing are due to the respiratory factor, and how far to a direct action.

Pituitary extract has a pronounced effect in constricting the bronchioles and this action is obtained even when the vagi are paralysed with atropine. Pituitary extract given to animals anæsthetised with a volatile anæsthetic or to pithed animals with an intact medulla leads

to an amplitude of respiratory movements, but also to a deficient oxygen content of the blood; this of course suggests that the increased amplitude of respiratory movements may be the result of partial asphyxia. The tracing Fig. 6 was obtained from a cat which was pithed and had the middle lobe of the right lung enclosed in an oncometer. It was receiving artificial respiration and at the time of the experiment the vagi had been paralysed by a previous injection of atropine. 1 c.c. of pituitary extract (Parke, Davis & Co.) suitably diluted injected into the femoral vein caused very decided constriction of the bronchioles. At first the constriction was complete, then air from the pump entered the lungs but the elastic recoil of the lungs was unable to expel it efficiently so that the curve rises and soon the constriction is complete, no air either entering or leaving the lungs.

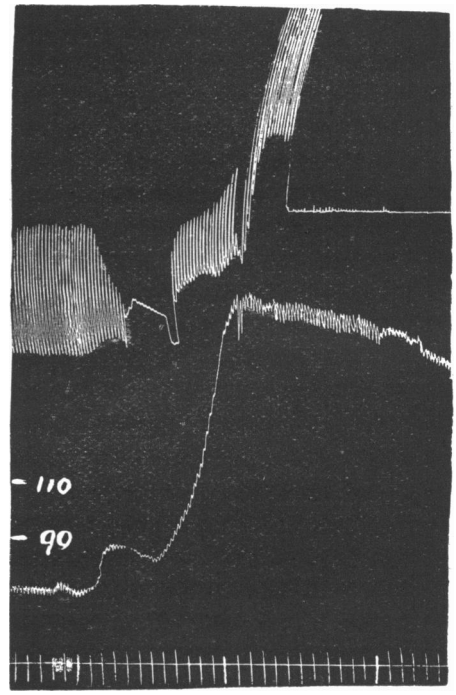

Fig. 6. Cat. Lung volume (upper tracing) and arterial pressure (lower tracing). Shows the effect of injecting 1 c.c. pituitary extract into a vein. Time = 5 secs.

Recovery was gradual and natural. In this case since the vagi were paralysed the action is probably on the muscular tissue, and it should be noted that this action occurred after each injection and was accompanied by increased respiratory movements as shown by the motion of the ribs and diaphragm. This typical experiment we have repeated on dogs with similar results.

We believe then that this action on the bronchioles explains the increased cerebro-spinal secretion, for although the respiratory efforts are increased they are largely unsuccessful, hence the blood becomes more venous. This indirect cause of increased cerebro-spinal secretion may be excluded first by employing urethane as the anæsthetic, and secondly by forceful artificial respiration. Urethane is in fact an ideal anæsthetic in such experiments, since it has a decided depressant action

on all forms of plain muscle. If urethane is employed, and artificial respiration is maintained at a constant and high level, the injection of pituitary extract has no effect whatever on cerebro-spinal secretion. For this reason we adhere to our previously expressed opinion that pituitary extract has no direct action on the secretory activity of the choroid gland.

Conclusions.

1. Substances introduced into the cerebro-spinal fluid which can be readily traced do not find an exit by the lymph channels, but appear rapidly in the blood.

2. If these substances are readily diffusible the speed with which they appear in the blood is very remarkable, especially if they are introduced into the subcerebellar region. An injection of substances like adrenaline, nicotine and atropine produces characteristic physiological effects almost as rapidly as if injected into the venous circulation.

3. Substances which are indiffusible such as proteins (Witte's peptone, serum in anaphylactic animals) do not produce their characteristic effects when they are introduced into the cerebro-spinal fluid. Whereas substances of intermediate molecular size, such as secretin, diffuse slowly and cause their characteristic actions at an intermediate rate.

4. The old theory that there are actual valved orifices leading into the large veins at the base of the brain is thus rendered quite untenable. The transference must be by diffusion.

5. This diffusion process is most rapid in the subcerebellar region, and is extremely slow in the lower spinal district. It probably occurs into the venous sinuses by the microscopic arachnoid villi described by Weed, but Mott's view that the transference partly occurs through the thin walls of the blood vessels within the central nervous system is not excluded by our experiments, seeing that contact of these vessels with the cerebro-spinal fluid is maintained throughout their extent by the perivascular spaces which are continuous with the subarachnoid cavity.

6. We further hold that this must also be the course taken by the normal cerebro-spinal fluid, and that it is by the blood and not by the lymph that the fluid which is constantly being secreted finds its main exit from the cranio-vertebral cavity.

7. We present, however, some evidence which shows that dyes added to the fluid travel along the course of certain cranial nerves,

especially the olfactory nerve. In the spinal nerves, no dye can be detected in their sheaths outside the spinal canal. No dye can be seen in the lymph of the thoracic duct.

8. Diffusion in the opposite direction from blood to cerebro-spinal fluid does not occur, except in an almost negligible degree in the case of a few drugs. The secreting epithelium of the choroid gland thus protects the tissue elements of the central nervous system from being bathed with harmful substances.

9. We further find in confirmation of our previous statement that pituitary extract has no direct action on the secretory activity of the choroid gland; the opposite statement by Weed and Cushing is explained by the non-exclusion in their experiments of an asphyxial condition (due to constriction of the bronchioles), which is a powerful stimulus to cerebro-spinal secretion.

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