Brit. J. Pharmacol. (1958), **13**, 156.

PERFUSION OF CEREBRAL VENTRICLES: EFFECTS OF DRUGS ON OUTFLOW FROM THE CISTERNA AND THE AQUEDUCT

BY

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(RECEIVED JANUARY 6, 1958)

In cats under chloralose anaesthesia the cerebral ventricles were perfused with Locke solution at a rate of 0.1 ml./min. from an indwelling cannula in the lateral ventricle. The effluent was collected from a cannula either inserted into the cisterna magna or pushed into the aqueduct. When collection was from the cisterna the perfusion included relatively large areas of the subarachnoidal spaces since in cats the foramina of Luschka form the only outlet from the fourth ventricle. Tubocurarine, histamine, and adrenaline injected intravenously caused great variations in outflow from the cisterna, but these changes did not occur when the collection was from the aqueduct. The changes in outflow from the cisterna were similar whether the injection produced a fall of arterial blood pressure as after tubocurarine and histamine, or a rise, as after adrenaline.

In the past, increased formation of cerebrospinal fluid (c.s.f.) by drug action was sometimes based on experiments in which an increased rate of leakage of c.s.f. from a cannula in the cisterna magna had occurred (Dixon and Halliburton, 1913; Weed and Cushing, 1915). As Davson (1956) points out, such an increased leakage does not necessarily signify increased secretion, ". . . for example, the normal drainage routes could be hindered by the effects of the drugs on the vascular pressures, and so on." In the present experiments the changes in flow of c.s.f. produced by drug action were observed by procedures which permitted differentiation between such effects and secretion from the choroid plexuses.

The methods used consisted of perfusion of the cerebral ventricular system in anaesthetized cats in such a way that the effluent could be collected from either the cisterna magna or the aqueduct of Sylvius. When the outflow was from the cisterna the perfusion fluid was in communication with the whole subarachnoid space, whereas when the outflow was from the aqueduct, perfusion was confined to the lateral and third ventricles. Changes in flow resulting from changes in secretion of the ventricular choroid plexuses are evident with both methods. Changes due to extrachoroidal c.s.f. formation, or passive adaptation in response to changes in the perivascular spaces of the brain, either do not occur, or occur to a slight degree only, when perfusion is confined to the lateral and third ventricles.

The present experiments deal with the effects of intravenous injections of tubocurarine, histamine and adrenaline, using both methods of collection.

The experiments were the outcome of work reported elsewhere (Bhattacharya and Feldberg, 1957, 1958) on the release of acetylcholine in the effluent from the perfused ventricles and on the appearance in this effluent of anticholinesterases injected intravenously. In these experiments it became necessary to inject tubocurarine intravenously in order to abolish muscular contractions, and it was found that these injections affected the outflow from the perfused ventricular system. Since tubocurarine releases histamine, and histamine releases adrenaline and noradrenaline from the suprarenals, the question arose as to whether the changes in outflow produced by tubocurarine were brought about indirectly by the release of these substances.

METHODS

The experiments were carried out in cats under chloralose anaesthesia. The cerebral ventricular spaces were perfused with Locke solution at a constant rate of inflow of 0.1 ml./min. from the cannulated left lateral ventricle. Fig. 1 is a diagrammatic representation of the two methods of collecting the outflow. The method of collection from the

cisterna magna, as illustrated in Fig. 1 (*a*), was the same as that described previously (Bhattacharya and Feldberg, 1957). In cats there is no foramen of Magendie, therefore the perfusion fluid passes from the foramina of Luschka at the lateral recesses of the fourth ventricle through a relatively large region of the subarachnoidal space before it reaches the cannula in the cisterna. This was shown when at the end of such a perfusion experiment methylene blue was injected through the ventricular cannula. It deeply stained the ventral part of the floor of the fourth ventricle, but not the caudal part beyond the lateral recesses ; however, the outer surface of the brain stem was deeply stained and the stain extended to the dorsal surface of the colliculi and to the ventral surface of the occipital pole of the cerebrum.

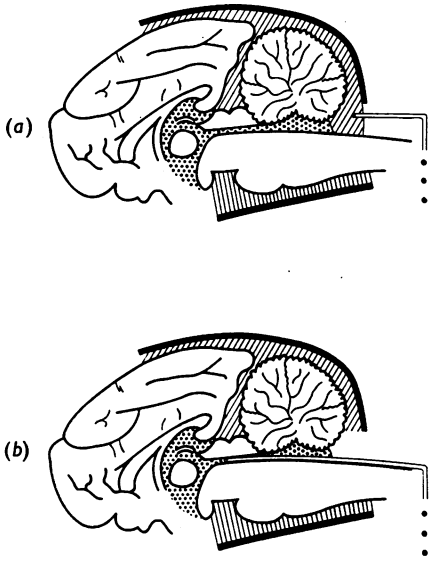

FIG. 1.—Diagrams of a median sagittal section of the brain of a cat, illustrating the two methods of collection of outflow during perfusion from the cannulated lateral ventricle. (*a*) Outflow from cisterna magna, (*b*) from the cerebral aqueduct. Dotted areas, ventricular spaces; shaded area, subarachnoidal spaces. The lateral ventricle is not shown. For details see text.

On the other hand, it is clear from diagram in Fig. 1 (*b*) that, when the cannula is in the middle of the aqueduct and fully fills its lumen, the perfusion is confined to the lateral and third ventricles and to the beginning of the aqueduct. The following procedure was adopted in order to insert the cannula into the aqueduct. The layers of muscle covering the atlanto-occipital membrane were dissected away and the membrane cut so as to expose the medulla. The lower part of the cerebellum was exposed by nibbling away the margins of the supra-occipital bone at the border of the foramen magnum. This made it possible to lift the cerebellum gently with a small curved spatula from the medulla and to insert a fine polythene tube along the floor of the fourth ventricle into the aqueduct. The outside diameter of this tube was 2 mm. and at its tip it was narrowed to 1 mm. The distance from the margin of the cerebellum to the middle of the aqueduct, where anterior and posterior colliculi meet, is 2 cm. or a little more. The tube was therefore inserted for at least 2 cm. beyond the margin of the cerebellum and the position of the tip of the cannula in the aqueduct was ascertained at the end of the experiments as follows. Either perfusion was stopped and 1 ml. of 1% methylene blue solution was slowly injected with a syringe through the intraventricular cannula, or the ventricular spaces were perfused for a few minutes with Locke solution containing methylene blue before the cat was killed. If the cannula was in the right position, the dye flowed out only through the polythene tube. If traces of dye seeped out around the tube, it meant that the tip of the cannula had not reached the aqueduct and that at least part of the fourth ventricle was not excluded from the perfusion. In this case the experiment was discarded. A further check was made after killing the cat by dissecting the brain without removing the tube and locating its tip in the aqueduct. With the cannula in the right position there was a sharp demarcation in the middle of the aqueduct between the heavily stained and the unstained tissue indicating the end of the perfused area.

To measure the outflow each drop was signalled on the smoked drum, or the intervals in seconds between each drop were noted. The outflow was collected in 10 or 20 min. samples, the total volumes of which were measured, and as the number of drops for each sample was counted, it was easy to convert the drops into fractions of a ml.

For intravenous injections the left femoral vein was cannulated. In some experiments, in which records of the arterial blood pressure were taken, the femoral artery of the other side was cannulated and connected with a mercury manometer. As anticoagulant, 0.5 to 1 ml. of 1% solution of heparin was injected into the arterial cannula. In those experiments in which tubocurarine was given, the cat was artificially ventilated through the cannulated trachea. For the injection, tubocurarine chloride, histamine acid phosphate and adrenaline chloride were used. All weights of histamine refer to the base, those of tubocurarine and adrenaline to the salt. The intravenous injections were made in 1 ml. solution washed in by 2 ml. saline solution.

RESULTS

When the cisterna had been cannulated, the spontaneous outflow of c.s.f. practically ceased when about 1 ml. had been collected ; perfusion with Locke solution was then started and the outflow collected in 20 min. samples. The rate of outflow, which at first was greater than the rate of inflow, gradually approximated to the rate of inflow. This is illustrated by the experiments in Table I, in which the outflow during the first

TABLE I

VOLUME OF OUTFLOW FROM THE CISTERNA DURING PERFUSION WITH LOCKE SOLUTION IN SUCCESSIVE 20 MIN. SAMPLES

Expt. No. 1	Expt. No. 2	Expt. No. 3
2·35	2·9	2·45
2·25	2·4	2·05
2·05	2·4	2·1
1·95	2·3	2·1
2·15	2·2	2·1
1·95	2·2	
	2·05	
	2·05	

20 min. sample exceeded the inflow by 0.35 to 0.9 ml. but slowed down in the following samples. In experiment No. 3, it approximated to the inflow during the second sample; in Experiment No. 2, only after 2 hr. of perfusion. The outflow showed irregular large fluctuations which were not dependent on respiration or on changes in arterial blood pressure. The experiments of Figs. 2 and 3 illustrate these fluctuations by differences of the interval in seconds between each drop. These fluctuations were not abolished when artificial ventilation was begun; the outflow then slowed down for a few minutes.

When the outflow was collected from the aqueduct, it was either equal to the inflow, or exceeded it by 0.05 to 0.15 ml. in 20 min., even under those conditions in which the cisternal outflow would have been much greater. The outflow from the aqueduct showed irregular fluctuations (Figs. 2 and 3), but they were often less pronounced than the fluctuations seen in the cisternal outflow.

In the experiments in which arterial blood pressure was recorded, 0.5 to 1 ml. of 1% heparin solution was injected into the blood pressure cannula. In several of these experiments the outflow became blood-stained after a time. Further, the injection of tubocurarine, histamine, and particularly of adrenaline, frequently led to the appearance of blood in the outflow. After a time the blood gradually disappeared from the effluent, only to reappear with a renewed injection. In some experiments the contamination with blood became so extensive as to give the impression that the outflow consisted mainly of blood; in this event the experiment had to be terminated. The contamination of the effluent with blood occurred whether the outflow was from the cisterna or from the aqueduct. It was found, post mortem, that the cannulated left lateral ventricle contained varied amounts of blood. The appearance of blood in the effluent was therefore attributed to bleeding from the injured tissue of the needle tract in the brain substance as a result of the heparin entering the circulation, and accelerated either by vasodilatation around the injured region produced by tubocurarine or histamine, or by the strong pressor action of adrenaline. The effects on the outflow described in the following paragraphs, however, were independent of this complication and the illustrations are from experiments in which the outflow was not contaminated with blood.

Tubocurarine.—The changes in outflow from the cisterna after an intravenous injection of 1 mg. of tubocurarine varied somewhat from experiment to experiment and also with repeated injections Usually, the injection caused first an acceleration in outflow, which after the first minute was interrupted by stoppage of the flow for a minute or even longer. During this time the flow not only stopped but the fluid was drawn back into the cannula, and a drop which had formed and was about to fall was seen to be drawn into the cannula. When the flow recommenced it soon became 2 or 3 times as fast as before the injection. This is illustrated in the experiment shown in Fig. 2, in which the time for the formation of each drop is plotted in seconds as ordinate, each vertical line representing one drop. The horizontal broken line indicates the rate of inflow; with the

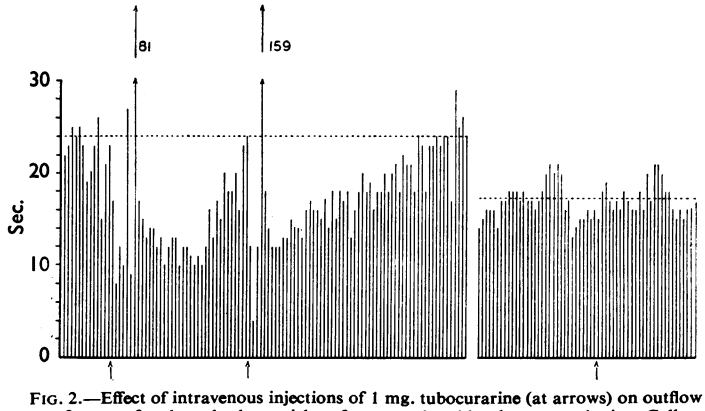

FIG. 2.—Effect of intravenous injections of 1 mg. tubocurarine (at arrows) on outflow from perfused cerebral ventricles of a cat under chloralose anaesthesia. Collection from cisterna on the left and from aqueduct on the right. Each vertical line represents one drop. Ordinate, interval between drops in sec. The numbers 81 and 159 refer to interval in sec. Horizontal broken line, rate of inflow (2 ml./20 min.). The diameter of the cannula used for collection from the aqueduct was smaller than from the cisterna, so that the volume of drops decreased and the time interval between drops was shortened. (For details see text.)

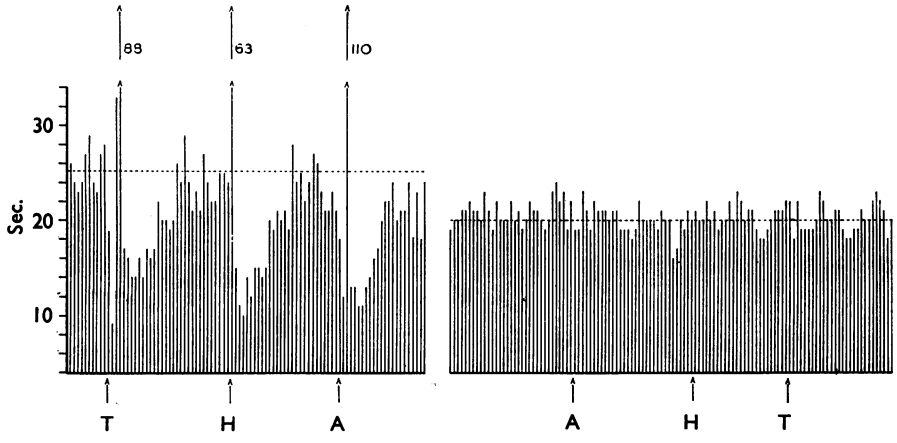

FIG. 3.—Effect of intravenous injection of 1 mg. tubocurarine (T), 10 μg. histamine (H) and 25 μg. adrenaline (A) on outflow from perfused cerebral ventricles of a cat under chloralose anaesthesia. Collection from cisterna on the left and from aqueduct on the right. Each vertical line represents one drop. Ordinate interval between drops in sec. The numbers 88, 63, and 110 refer to intervals in sec. Horizontal broken line, rate of inflow (2 ml./20 min.). The diameter of the cannula used for collection from the aqueduct was smaller than from the cisterna, so that the volume of drops decreased and the time interval between drops was shortened. (For details see text.)

cannula used it was equivalent to an outflow of one drop every 24 sec. Before the injection the outflow was a little in excess of the inflow as shown by the fact that most of the intervals were less than 24 sec.; during the 10 min. preceding the injection 1.1 ml. was collected. The two injections of 1 mg. tubocurarine which followed each other at 10 min. intervals produced each time an acceleration of outflow for 3 to 4 drops followed by a slowing down or stoppage of the outflow, so that the interval for one drop became 81 sec. after the first and 159 sec. after the second injection of tubocurarine. The outflow then quickly increased, remained high for a few minutes and then returned gradually to the pre-injection rate of flow. The overall effect was an increased outflow which amounted to 1.4 ml. in 10 min. after the first and to 1.3 ml. in 10 min. after the second injection; it then became practically normal again and amounted to 1.15 ml. in the next 10 min. sample. This experiment thus illustrates that intravenous tubocurarine can cause an increased outflow from the cisterna. In other experiments in which the injection of tubocurarine produced similar variations in outflow from the cisterna, the overall effect was small and sometimes even absent. For instance, in the experiment shown in Fig. 3 the intravenous injection of 1 mg. tubocurarine caused the same variation, acceleration, slowing down and acceleration of outflow; the overall effect showed scarcely any increase in outflow because 1.1 ml. was collected in the first 10 and 1.7 ml. in the first 15 min. after the injection, as compared with 1.05 to 1.1 ml. in preceding 10 min. samples.

When collection was from the aqueduct the intravenous injection of 1 mg. tubocurarine produced practically no change in outflow, whether the tubocurarine had increased the overall outflow from the cisterna or not (Figs. 2 and 3).

Fig. 4 (*a*) and (*b*) illustrates two experiments in which the blood pressure from the femoral artery was recorded. The initial acceleration of outflow from the cisterna which followed the intravenous injection of 1 mg. of tubocurarine occurred before the main depressor effect. This depressor effect was preceded by a small pressor effect. A similar but even smaller rise of blood pressure was produced by an intravenous injection of saline solution. The initial acceleration in outflow from the cisterna coincides with this rise in blood pressure. When the outflow begins to slow down or to stop, the blood pressure may still be elevated or it may already have begun to fall. If the slowing is only transient as in experiment Fig. 4(*a*), the subsequent acceleration begins whilst the pressure is at its lowest level. In experiment Fig. 4(*b*), on the other hand, the stoppage of outflow lasted longer and the subsequent acceleration of outflow began after the blood pressure had practically recovered and a secondary more sustained fall had occurred. When the injections of tubocurarine were repeated at 10 or 20 min. intervals, it was often observed that after a few injections the effects on the out-

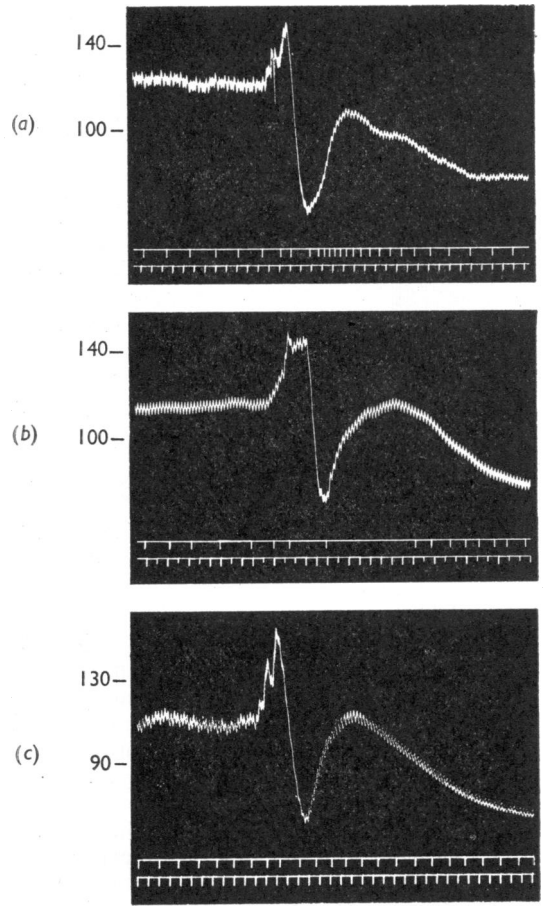

FIG. 4.—Effect of intravenous injections of 1 mg. of tubocurarine on arterial blood pressure and outflow from the perfused cerebral ventricles of three cats under chloralose anaesthesia. Each signal in the middle trace represents one drop. In cats (*a*) and (*b*) outflow was collected from the cisterna, in cat (*c*) from the aqueduct. Time (lowest trace), 10 sec.

flow diminished and were sometimes very small. When this happened the tubocurarine injections produced much smaller depressor effects, probably as the result of the diminished histamine release with the repeated injections.

Fig. 4(*c*) shows that, when collection was from the aqueduct, the intravenous injection of tubocurarine no longer affected the outflow although it caused pronounced changes in blood pressure. In this experiment there was a slight acceleration of outflow which was not regularly observed and was within the limit of the normal fluctuations.

Histamine.—The changes in outflow from the cisterna after an intravenous injection of 10 μg. of histamine varied somewhat from experiment to experiment, but resembled those described for tubocurarine. In the experiment shown in Fig. 3 the injection caused a stoppage of outflow followed by pronounced acceleration, but there was scarcely any overall increase. In other experiments the changes in outflow consisted of acceleration followed by retardation, or of an initial short retardation followed by acceleration which in its turn was followed by retardation. These results are illustrated in Figs. 5 and 6 in which the arterial blood pressure was also recorded. In both experiments the increased outflow started after the blood pressure had fallen and continued during its recovery. When this acceleration was preceded by some retardation in outflow as in Fig. 5, the retardation occurred whilst the pressure was falling. The late reduction in outflow seen in Fig. 6 occurred after the blood pressure had risen. In some experiments the overall effect resulted in an increased outflow of about 0.1 ml. during the 10 min. following the injection ; in others, there was no overall increased outflow.

Figs. 3 and 5(*b*) show that histamine did not cause these changes in outflow when collection was from the aqueduct.

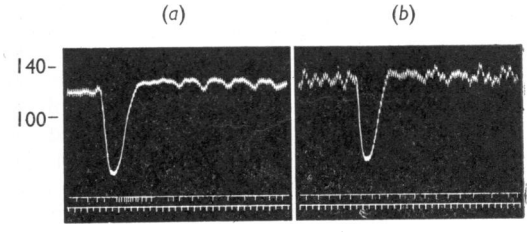

FIG. 5.—Effect of intravenous injections of 10 μg. of histamine on arterial blood pressure and outflow from the perfused cerebral ventricles of a cat under chloralose anaesthesia. At (*a*) outflow collected from cisterna, at (*b*) from aqueduct. Each signal in the middle trace represents one drop. Time, 10 sec.

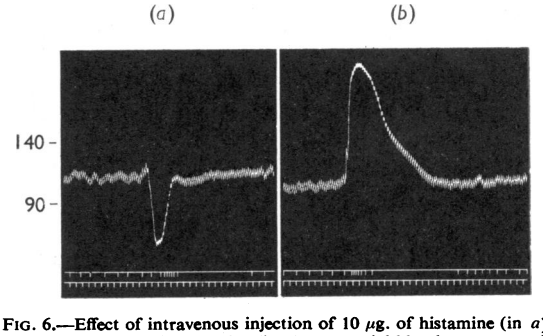

FIG. 6.—Effect of intravenous injection of 10 μg. of histamine (in *a*) and of 25 μg. of adrenaline (in *b*) on arterial blood pressure and outflow from the cisterna during perfusion of the cerebral ventricles of a cat under chloralose anaesthesia. Each signal in the middle trace represents one drop. Time, 10 sec.

Adrenaline.—Although intravenous injection of adrenaline caused a rise in blood pressure, the changes in outflow from the cisterna resembled those seen after histamine or tubocurarine (Figs. 3 and 6). In Fig. 3 the intravenous injection of 25 μg. adrenaline caused changes in outflow similar to those caused by a previous injection of 1 mg. tubocurarine. There was initial acceleration and then stoppage of the flow for nearly 2 min. followed again by a period of acceleration. In the experiment of Fig. 6 the arterial blood pressure was recorded also. The changes in outflow from the cisterna produced by adrenaline were similar to those produced by histamine although histamine caused a fall and adrenaline a rise in pressure. The acceleration of outflow which followed the injection of adrenaline coincided with the rise of pressure ; the stoppage of flow occurred when the pressure was falling again. Adrenaline always caused a prolonged stoppage of outflow whereas with tubocurarine and histamine the stoppage was sometimes transient. In some experiments with adrenaline the stoppage of flow was followed by some slight but definite acceleration as in Fig. 6.

These changes produced by adrenaline in the outflow from the cisterna caused either no overall effect in outflow or a slight increase.

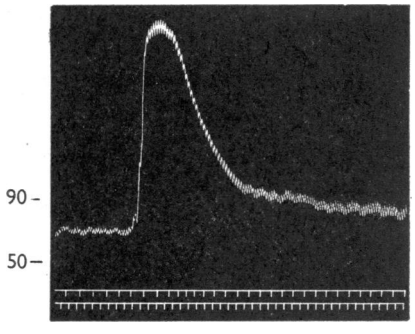

FIG. 7.—Effect of intravenous injection of 25 μg. of adrenaline on arterial blood pressure and outflow from the aqueduct during perfusion of the cerebral ventricles of a cat under chloralose anaesthesia. Each signal in the middle trace represents one drop. Time, 10 sec.

When the cannula was in the aqueduct the intravenous injection of 25 μg. of adrenaline did not produce the characteristic changes in outflow observed during collection from the cisterna. In some experiments, as in that shown in Fig. 7, there was a slight and transient acceleration of outflow during the rise in blood pressure ; in other experiments, the injections had no effect on the outflow.

DISCUSSION

The results of the present experiments on the perfused cerebral ventricles of the cat show that variations in outflow from the cisterna magna produced by intravenous injections of tubocurarine, histamine, and adrenaline cannot be attributed to secretion of c.s.f. from the choroid plexus of the lateral and third ventricles because the variations do not occur when the outflow is collected from the aqueduct. They must therefore be attributed to changes which occur either in the fourth ventricle or in the subarachnoidal space. As it is unlikely that the choroid plexuses of the fourth ventricle react to drugs differently from the choroid plexus of the third and lateral ventricles, the observed variations in cisternal outflow appear to be dependent on the inclusion of the subarachnoidal space in the perfusion system.

In the analysis of variations in cisternal outflow, a distinction must be made between variations in the production of c.s.f. and variations in the capacity of the subarachnoidal space resulting from blood pressure changes or from effects on the vessels covering the meninges. To a large extent the observed variations in cisternal outflow reflect adaptations in response to changes in the perivascular spaces of the central nervous system. This conclusion is based on the observation that acceleration was often followed by slowing or stoppage of the outflow and *vice versa,* so that the changes were to a great extent compensated. Further, when stoppage of outflow occurred, a drop which was partly formed was often seen to be drawn back into the cannula ; this indicated a transient increase of the available subarachnoidal space.

In some experiments adrenaline, which caused a rise in blood pressure, and histamine, which caused a fall, both produced an acceleration followed by slowing of the cisternal outflow. The acceleration after adrenaline could be explained by reduction of the subarachnoidal space brought about by an increase in the volume of the vascular bed in the meninges, due either to passive dilatation to the rise in arterial blood pressure, or to a dilator effect of adrenaline on the meningeal vessels. The subsequent slowing or stoppage of outflow which occurred when the blood pressure fell again could be the result of the re-establishment of the original capacity of the subarachnoidal space. On the other hand, the acceleration of outflow which occurred in the same animal after an intravenous injection of histamine could be the result of a vasodilator action of the histamine on the meningeal vessels. As long as the blood pressure

was low the reduction in subarachnoidal space due to this vasodilatation was compensated by the fall in arterial blood pressure. The acceleration in outflow commenced only when the blood pressure recovered, and the subsequent reduction or stoppage in outflow may represent the disappearance of the histamine vasodilatation in the meninges. The finding that blood pressure changes as well as vascular changes in the meninges affect the outflow, and sometimes in the opposite direction, would account for the fact that in different experiments the drugs affected the cisternal outflow somewhat differently.

Although the variations in cisternal outflow produced by the intravenous injections of drugs were to a large extent accounted for by changes in the relative capacity of the subarachnoidal space, there was also evidence for an increased production of c.s.f. In several experiments the volume of fluid collected from the cisterna during a 10 or 20 min. period after an intravenous injection of tubocurarine was greater than the inflow and greater than the volume collected during a similar period before the injections. This also happened to a lesser degree after an intravenous injection of histamine or adrenaline, and the only explanation would be that the production of c.s.f. had increased. Since the increased outflow did not occur under otherwise similar conditions when collection was from the aqueduct, it cannot be attributed to increased secretion from the choroid plexus of the lateral and third ventricles, and it is unlikely that the choroid plexus of the fourth ventricle would react differently from those of the lateral and third ventricles. The increased c.s.f. production is probably non-choroidal in origin, taking place in the subarachnoidal space. It may enter the subarachnoidal space by diffusion either from the brain substance or from the vessels of the meninges. This recalls observations of MacIntosh and Oborin (1953), who found that fluid transudes from the cat brain to a saline pool created at the surface of the cerebral cortex.

References

Bhattacharya, B. K., and Feldberg, W. (1957). *J. Physiol.*, **135**, 4P.

—— —— (1958). *Brit. J. Pharmacol.*, **13**, 163.

Davson, H. (1956). *Physiology of the Ocular and Cerebrospinal Fluids.* London: Churchill.

Dixon, W. E., and Halliburton, W. D. (1913). *J. Physiol.*, **47**, 215.

MacIntosh, F. C., and Oborin, P. E. (1953). *Abstr. XIX int. physiol. Congr., Montreal*, p. 580.

Weed, L. H., and Cushing, H. (1915). *Amer. J. Physiol.*, **36**, 77.