Secretion of cerebrospinal fluid by choroid plexus of the rabbit

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Blood flow in the principal draining vein of the choroid plexus of the lateral ventricle of the rabbit, which drains approximately the anterior 70% of the plexus, was measured by the analysis of motion pictures made during the injection of spheruler of 1-octanol. The relative loss of volume from the plasma compartment during the transit of blood was measured by the comparison of relative cell volumes in aortic blood and in venous blood of the plexus. The blood flow of the plexus averaged 2.86 μl/mg min and the rate of production of cerebrospinal fluid .37 μl/mg min. The effect upon the relative rate of production of fluid of several inhibitors was tested and of these acetazolamide was effective both intravascularly and topically; cardiac glycosides were effective topically, but not when given intravenously, and cyanide and 2,4-dinitrophenol were ineffective topically.

For more than a hundred years the hypothesis that cerebrospinal fluid is formed by the choroid plexus has been widely, if not universally, accepted. Suggested by Faivre in 1853 when he discovered that the ependymal covering of the plexus has a glandlike structure, the choroidal origin of the fluid has been neither rigidly proved nor effectively challenged. The clinical occurrence of hydrocephalus behind a ventricular block, the creation of experimental hydrocephalus by Dandy and Blackfan (5), and Frazier and Peet (14), and especially the demonstration by Dandy (4) that the ventricular distention which inevitably occurred proximal to a blocked foramen of Monro did not ensue on one side when the plexus was removed from that ventricle, were considered to approach proof. Bering (1) has recently cast doubt upon the interpretation of these observations. He has demonstrated that of lateral ventricles communicating with each other, but behind a block, only the ventricle containing choroid plexus became distended while that deprived of plexus did not. The phasic difference of pressure between fluid and brain occasioned by the pulsation of the plexus was considered to be the essential factor.

During recent years there have been several new approaches which might be capable of testing the hypothesis. The first is the exceptional experiment of de Rougemont et al. (9) who sampled and analyzed the film of fluid over the choroid plexus. Although the fluid they analyzed was shown to be newly formed and it seems extremely likely that it arose from the plexus, it is not absolutely certain that it was not of extrachoroidal origin. Another is the isolated preparation of choroid plexus devised by Howarth and Jowett (17) and by Clark (3). If it can be shown that in this there is no connection between the artificial sac and the ventricle or meninges, a choroidal source of fluid will be established.

The approach to be presented here involves estimation of the blood flow of the choroid plexus and the loss of volume from the plasma during the transit of blood.

The vascular arrangements of the choroid plexus of the lateral ventricle of the rabbit are favorable for the direct measurement of venous blood flow, as the venous drainage is largely through one vein. According to the observations of Millen and Woollam (27), with which our own are in accord, one or several smaller veins leave the plexus through the choroidal fissure but the principal vein of the choroid plexus passes for the greater part of the length of the structure, gathering tributaries as it courses toward the foramen of Monro, and finally passes through this foramen. The vessel is not difficult to expose and measure as it approaches the foramen and estimates of average linear velocity of its blood can be made from motion pictures of the passage of a suitable interface introduced into the vessel by puncture. It is also not difficult to obtain samples of the venous blood of the plexus and to compare the hematocrit of this blood with that of blood obtained from the aorta.

If, during the transit of blood through the choroid plexus, the erythrocytes neither appreciably swell nor shrink, the volume of erythrocytes entering and that leaving the plexus during any period are necessarily equal.

Hct,Qa = Hct,Qv

Received for publication 25 March 1963.

1 This study was aided by Research Grant B-2931 from the National Institute of Neurological Diseases and Blindness.
where Hct = hematocrit, Q = flow, and the subscripts \(a, v, \) and csf refer to arterial, venous, and cerebrospinal fluid, respectively.

The difference between the arterial flow and the venous return, that is the volume of cerebrospinal fluid formed per unit time is

\[
Q_a - Q_v = Q_{csf} = Q_v \left( \frac{Hct_v}{Hct_a} - 1 \right)
\]

An attempt is made to specify \(Q_v, Hct_v,\) and \(Hct_a\), and to show that the condition given, little or no change in average erythrocyte volume, is reasonably well met.

The effect of several conditions upon the relative rate of production of fluid also has been tested and in this connection, the fraction of arterial flow lost as fluid, \(1 - \frac{Hct_a}{Hct_v}\), is stated.

**METHODS**

**Measurement of venous blood flow.** Adult male rabbits were anesthetized by the intraperitoneal administration of urethane, \(1.8 \text{ g/kg}\), and a tracheostomy was made. A vinyl tube of \(.5 \text{ mm internal diameter}\) was filled with heparinized saline and threaded through one femoral artery into the aorta. The tube was attached to a strain gauge and arterial pressure was recorded continuously by a pen-writing device.

The head was mounted in a specially designed and fashioned head holder attached to the base stand of the Leitz micromanipulator. The anterior portion of the animal's head was closest to the operator. A craniectomy was made and the dura mater opened. Under the low power of the Leitz dissecting microscope, the cortex and underlying white matter of the superior parts of both cerebral hemispheres were removed and the lateral ventricles were entered. After hemostasis was achieved, heparin, \(3,000 \text{ I.U.}\), was given intraperitoneally, or one-sixth this amount by vein.

Employing a microneedle held in the right-hand micromanipulator, the free edge of the left choroid plexus was penetrated and the plexus reflected backward. This exposed the anterior part of the main choroidal vein and its tributaries and also provided countertraction during puncture of the vessel. The exposure of the plexus involved only its anteriormost part. The major portion was hidden from view and was bathed in cerebrospinal fluid.

A micropipette of \(25 \mu\text{m tip size} \) held in the left-hand manipulator was attached by vinyl tubing to a gas-tight syringe containing 1-octanol. The pipette was filled with the octanol and a .9% saline solution was then drawn into the pipette so as to occupy a centimeter or so of its distal shaft. This protected the plexus from any deleterious effects of the octanol during puncture and a small test injection.

By the use of an eyepiece micrometer standardized against a stage micrometer, the diameter of the vein was measured as was a length between convenient and easily recognized landmarks.

The micropipette was then introduced into the vein. The right ocular lens of the microscope was removed and a motion picture was made through that eyepiece tube during injection of the octyl alcohol. Ordinarily, gentle pressure on the piston of the syringe resulted in the passage into the vein of a train of spherules of the same diameter as that of the vessel at a rate of one to several per second. The plexus often became congested afterward due to thrombosis of the vein.

The motion picture camera was a Kodak Reflex Special. An aluminum tube was made to fit the lens-retaining device of the camera. The projecting portion of the tube was 6.5 cm in length and 2 cm in diameter. The camera was mounted on a Kodak high-speed camera stand. It was powered by a variable-speed motor manufactured by Camera Equipment Company, New York. The speed of the motor was determined by repeatedly clocking the passage of 3,000 frames after full acceleration had been reached. The speeds employed were between 65 and 85 frames/sec and were known within narrow limits, the standard deviation being approximately .15%.

For making the motion pictures the aluminum tube attached to the lens-retaining device of the camera was lowered into the eyepiece tube of the dissecting microscope and focus was achieved by the focusing screw of the microscope. Illumination was from a Leitz Monla...
lamp held close to the plexus. Superansochrome film was employed. After the filming was completed the plexus was removed, gently blotted, and weighed.

The film was projected on graph paper, the segment of the vessel which was of interest being superimposed on one of the lines of the paper. Lines were drawn on the paper to identify the landmarks limiting the course which was measured earlier and individual frames were then projected, the position of the center of a spherule being marked and the frame number recorded. The linear velocity was taken as the measured distance of the entire course times the fraction of the course traversed in the number of frames required for that passage, divided by the time required for the exposure of that number of frames. Alternatively, the marks identifying the center of the spherules were displaced at a right angle to the direction of flow a specified distance for each frame projected. This gave a linear plot the slope of which corresponded to the linear velocity (Fig. 1). The interval of time between the appearance at a specified point of the measured spherule and the following one also was noted.

The blood flow in the main choroidal vein, $Q$, was taken as the cross-sectional area of the vein, $\pi r^2$, times the linear velocity, $\frac{1}{t}$, less the volume of octyl alcohol injected, $\frac{4}{3}\pi r^2 n$, where $n$ is the number of spherules injected during one unit of time.

$$Q = \pi r^2 \frac{1}{t} - \frac{4}{3} \pi r^2 n$$

Proportion of choroid plexus drained by main choroidal vein.
It has been pointed out by Hudson (18) that in several mammalian species the major portion of the venous drainage of the plexus is by the main choroidal vein into the internal cerebral vein but that one or several smaller veins drain the posterior extremity of the plexus via the choroidal fissure into the basal vein. In order to relate blood flow to weight it is of some importance to define, if possible, the relative proportions of the plexus drained by each system. If the choroid plexus is gently teased out of the ventricle forward and medially toward the foramen of Monro while under observation through the dissecting microscope, a watershed between the two venous drainages can be made out. In 17 instances the plexus was cut at this point and the two portions of the plexus weighed.

Sampling of blood and treatment of samples. For sampling of blood from the choroidal vein a tip of approximately 30-50 $\mu$ diameter is best. It is often most convenient to employ a smaller tip for the initial puncture and by gentle advancement of the pipette to dilate the hole in the vein. Larger tips can then be easily and repeatedly introduced through the same opening in the wall of the vein. Aggregations of platelets which form at the site of puncture can be dislodged by touching the vein and often become less of a nuisance as time passes.

Hematocrit. Venous blood from the plexus and blood from the aorta were collected in capillary tubes which were sealed in a flame. The arterial and venous capillaries were centrifuged simultaneously for 5 min at 11,000 rev/min in a microcapillary centrifuge (manufactured by International Equipment Co., Boston). The hematocrits were read by pasting the capillaries on a ruler marked at .1-in. intervals and inspecting them under the dissecting microscope.

Erythrocyte count. Erythrocyte counts were made after taking samples of venous blood into a capillary and marking the top of the column of blood with Scotch tape. The blood was then washed into a 10-ml volumetric flask with 9% saline and the pipette set aside for later calibration. The contents of the flask were brought up to the mark and a further dilution of 1/50 was made. Five microliters of blood from the arterial catheter were similarly treated and counts of fluid from arterial and venous flasks were made with the Coulter counter (manufactured by Coulter Electronics, Chicago). The mean of at least three counts of .5-ml samples was subjected to correction for coincident passage and the counts were related to volume and to each other.

Calibration of the pipette was made by aspirating into the washed and dried capillary, to the mark, a solution of radiiodinated rabbit serum in a strongly alkaline solution, and expressing and washing this into a counting tube. Counts obtained in a well-type scintillation detector were compared to those of a measured volume of the same solution.

CO$_2$ content. Arterial blood was gently withdrawn under oil and the CO$_2$ content determined at least twice, employing the Natelson manometric gasometer (28).

| TABLE I. Blood flow of the choroid plexus and rate of production of cerebrospinal fluid |
|-----------------|-----------------|-----------------|-----------------|-----------------|------------------|
| Exp. No.  | Flow, m.c.v., $\mu$l/min  | Wt. Plexus, mg  | Wt. Plexus Drained, mg  | Art. Blood, CO$_2$, ml/mg min  | CSP Rate of Production, ml/mg min |
| F-16    | 26.36  | 5.48  | 2.77  | 3.12  |
| F-17    | 22.93  | 6.30  | 4.20  | 1.90  | 7.48  |
| F-20    | 23.71  | 5.20  | 3.14  | 1.50  | 3.10  |
| F-24    | 14.48  | 7.14  | 5.10  | 1.16  | 3.10  |
| F-21    | 3.62   | 6.50  | 4.50  | 1.10  | 3.20  |
| F-25    | 13.27  | 6.15  | 4.04  | 1.20  | 3.20  |
| F-29    | 12.39  | 5.45  | 3.75  | 1.07  | 3.97  |
| F-30    | 20.23  | 7.54  | 7.43  | 1.28  | 4.30  |
| F-31    | 6.56   | 7.89  | 4.35  | 1.09  | 1.47  |
| F-32    | 9.22   | 5.49  | 3.93  | 1.09  | 2.53  |
| F-33    | 8.86   | 6.50  | 5.11  | 1.13  | 1.83  |
| F-34    | 9.39   | 6.50  | 5.10  | 1.20  | 1.73  |
| F-35    | 3.27   | 6.88  | 4.49  | 1.18  | 1.88  |

Mean  | 11.43  | 6.46  | 4.16  | 2.86  | 4.67  |

* For F-16 through F-32 the individual weights were multiplied by .715, the mean fraction of plexus drained through the main choroidal vein. For F-33 through F-35 the fraction was individually determined.
Venous samples were taken under mercury, and the blood and some of the mercury were transferred to the intake pipette of the apparatus. The column of blood was taken up until its lower edge was at the .01 mark and its volume was estimated by measurement of its length. It had previously been shown by measurement of a column of mercury at a number of points along the portion of the tube between the .01 and the .02 marks that the lumen of the pipette was of uniform bore. Analyses were carried on from this point as though for a larger sample.

Uncertainties and errors. Certain assumptions have been necessitated by the lack of precise methods of assessment. These are:

1) That the main choroidal vein has a circular cross section. The vessel appears under the binocular microscope to be approximately cylindrical, but small differences in the two axes might be overlooked with consequent inaccuracy in estimation of cross-sectional area.

2) That the presence of the tip of the pipette within the vessel does not appreciably interfere with flow. It obviously does, and the extent to which venous blood is diverted to channels, the flow in which is not measured, cannot be satisfactorily estimated.

By avoiding the measurement of events during acceleration of octanol spherules or of the camera, or during the passage of small spherules traveling in the axial stream or of cylinders of the octanol, matters which have been considered by McDonald (26), errors can be minimized.

The SD of measurement of the radius of the main choroidal vein is estimated at .005 mm as the diameter was read to the closest mark of the micrometer scale at each side of the vessel, the intervals of the scale being .017 mm. The SD of measurement of length is estimated to be .038 mm, allowing for a range between marks at each end of the measured distance, the scale intervals in this case being .067 mm.

The SD of the hematocrit by the method employed was determined by the analysis of some 300 measurements to be .53 of a hematocrit unit.

The SD for the expression

$$\left( \frac{Hct_v}{Hct_a} - 1 \right)^{\frac{1}{2}}$$

was calculated to be approximately 12% of the value.

By repeated determinations on 10-μl samples of the same blood with a mean value of CO₂ of 14.6 mEq/liter a SD of 1.6 mEq/liter was found.

RESULTS

Relation between hematocrits of choroidal venous and aortic blood. The mean value of the ratio of hematocrits of choroidal venous to aortic blood in 67 rabbits was 1.15 ± .008 (mean ± SEM). The findings reflect an average loss of 13.3% of blood volume during transit. The mean relative cell volume in these 67 instances was 47.66 ± .67%.

Ratio of hematocrit of blood from a superior cortical vein to that from the aorta. In seven animals blood from a superior cortical vein was sampled and the relative cell volume of this compared to that of blood from the aorta. The ratio of venous to arterial hematocrits was 1.03 ± .01 (mean ± SEM).

Comparison of erythrocyte counts of choroidal venous blood and aortic blood. In eight animals samples of choroidal venous blood were taken serially for determinations of hematocrits and of erythrocyte counts. In these the mean was obvious during the sampling. The animal which received .77 mg/kg exhibited a severe toxic reaction with vomiting and labored breathing and succumbed shortly after the second blood samples were taken.

FIG. 2. Effect of topically applied acetazolamide on relative rate of secretion of cerebrospinal fluid. Each point was derived from the ratio of the mean hematocrit of 8-10 arterial samples to the mean of duplicate venous samples. The limits are the SDs of the ratios. The determinations at zero time are control values in this and subsequent figures.

FIG. 3. Effect of digitoxin, given intravenously, on the relative rate of elaboration of cerebrospinal fluid. Points were derived as described in legend of Fig. 2. The apparent increases are associated with and undoubtedly due to diminished blood flow which was obvious during the sampling. The animal which received .77 mg/kg exhibited a severe toxic reaction with vomiting and labored breathing and succumbed shortly after the second blood samples were taken.

FIG. 4. Effect of topically applied ouabain on relative rate of production of cerebrospinal fluid. Derivation of points and limits is as described in legend of Fig. 2. In a concentration of 10⁻³ M, secretion was severely inhibited. It was approximately halved in the presence of 10⁻² M ouabain.
ratio of venous to aortic hematocrits was 1.15 ± .03 (mean ± SEM), while the ratio of erythrocyte counts was 1.14 ± .02 (mean ± SEM). The average difference between the ratio of counts or of hematocrits and their mean was 1.9%, the range being between 0.5 and 4.5%.

Weight of choroid plexus. The mean (±SEM) weight of the choroid plexus of both lateral ventricles and the fourth ventricle in 45 rabbits was 23.4 ± .5 mg. This figure is considered to be more reliable than one published earlier (33), as the earlier figure was determined by adding the weights of a number of fragments of each plexus and was based upon less experience in the removal of the plexus in toto. In the 45 instances the mean weight of the choroid plexus of the fourth ventricle, 7.87 ± .2 mg, represented one-third the total.

Proportion of plexus drained by main choroidal vein. By cutting the plexus at the clearly visible divide between the anterior and posterior venous drainages and weighing the fragments separately, the proportion of plexus drained anteriorly into the main choroidal vein was determined to be 71.5 ± 1.8% (mean ± SEM, 17 plexuses).

Blood flow of choroid plexus and rate of production of cerebrospinal fluid by plexus. In Table 1 are summarized experiments in which venous return from the choroid plexus was estimated after sampling blood for the determination of hematocrits. Excluded are measurements made after a fall of more than 10 mm Hg in diastolic pressure as this seemed to affect the flow. Venous return in three such instances averaged only .94 µl/mg min. While this series of experiments was being made measurements could not be successfully concluded in three animals, in one because there were two veins rather than one passing almost to the foramen of Monro, in another because a thick coat of connective tissue over the vein made its puncture impossible, and in a third because of failure of the camera.

Examination of these 16 experiments shows that in several respects the sample is not representative of rabbits in general. Although the mean of the ratios of venous to arterial hematocrits in this group, 1.16, is almost the same as that of the larger group cited earlier, the spread is greater than expected and the distribution is somewhat lopsided. Also, the mean weight of the choroid plexus of the lateral ventricle is less than that expected, although in this instance a systematic difference has unfortunately been introduced in that in these experiments the tare was weighed after the combined tare and plexus, and in the larger series the tare was weighed before the two.

From these data the choroidal source of cerebrospinal fluid also can be estimated with respect to the weight of the plexus, and the mean choroidal contribution for one lateral ventricle, which should be one-third the total, is approximately 2.6 µl/min. The total choroidal production should, therefore, be 7.8 µl/min.

Effect of acetazolamide. After the intravascular administration of acetazolamide, ordinarily through a catheter into the vena cava, it was observed that the movement of fluid medially in the declivity between the hippocampus and caudate nucleus, ordinarily visible under the dissecting microscope because of the presence in the fluid of suspended erythrocytes, ceased, and the pooling of fluid in the anterior part of the ventricle no longer occurred. The exposed portion of the plexus, which ordinarily remained moist, tended to dry out. In ten animals the mean fraction of blood flow lost as fluid was reduced from a control value of .14 ± .01 (mean ± SEM) to .02 ± .02 30 min after administration of 75–100 mg/kg acetazolamide. The effect was neither prevented nor attenuated by varying atmospheres of CO₂ provided by bathing the plexus in Tyrode’s solution equilibrated with the varying concentrations of CO₂. During the minute or so required for taking the subsequent sample, another irrigation with equilibrated Tyrode’s solution was carried out and the CO₂ was continually released close to the plexus in the necessarily uncovered field.

With topically applied acetazolamide (Fig. 2), the inhibition was severe when the bathing solution contained 10⁻³ M acetazolamide. At 10⁻⁴ M the rate of production was approximately halved.

Carbon dioxide content. Because the exchange of CO₂ across the choroid plexus would have a bearing upon the ion-exchange hypothesis proposed by Tschirgi et al. (33), the CO₂ content of arterial blood and of venous blood of the choroid plexus was measured (Table 2). The content in venous blood was determined on samples of 5–15 µl. The levels of CO₂ in the venous blood fell far short of those anticipated on the basis of the ion-exchange hypothesis.

Effect of cardiac glycosides. Although the intravenous administration of digitoxin was not followed by a depression in the relative rate of secretion of fluid (Fig. 3), the topical application of ouabain consistently reduced the rate of its production (Fig. 4). The effect, in the presence of 10⁻⁵ M ouabain, was to approximately halve the rate, while a bath containing 10⁻⁴ M ouabain almost completely inhibited the elaboration of fluid.

That digitoxin was ineffective by vein may be only a reflection of dosage. More could not be given and one animal succumbed to a dosage of 77 mg/kg, fortunately not before blood samples were obtained.

Other inhibitors. The topical application of CN⁻ (Fig. 4) and 2,4-dinitrophenol (Fig. 5) failed to produce a TABLE 2. CO₂ content of whole arterial blood and venous blood of choroid plexus

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Fraction of Blood Lost at CSF</th>
<th>CO₂ Aortic Blood</th>
<th>CO₂ Venous Blood</th>
<th>Venous CO₂ Predicted by Ion-Exchange Hypothesis * mEq/l</th>
<th>mEq/l</th>
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<tr>
<td>C 1</td>
<td>.01</td>
<td>11.4</td>
<td>15.4</td>
<td>30</td>
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<tr>
<td>C-2, AM</td>
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<td>18.0</td>
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<tr>
<td>C-3, PM</td>
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<td>19.5</td>
<td>24</td>
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<tr>
<td>C-3</td>
<td>.11</td>
<td>16.4</td>
<td>22.0</td>
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<tr>
<td>C-4</td>
<td>.14</td>
<td>17.7</td>
<td>23.2</td>
<td>42</td>
<td>42</td>
</tr>
</tbody>
</table>

* \[
\frac{\text{Hct}_{\text{Venous}}}{\text{Hct}_{\text{Arterial}}} \times \text{Estimated Na+ concentration in nascent fluid (150 mEq/liter) + CO₂ arterial blood.}
\]
consistent difference in the relative rate of production of fluid.

**DISCUSSION**

**Blood flow of the choroid plexus.** Although the limitation which the blood flow of the choroid plexus places upon the activity of that gland in the elaboration of cerebrospinal fluid has been realized, and a prescient estimate of this has been forwarded by Heisey et al. (16), there has been no other study of that flow. The arterial flow, which has been estimated to average 2.80 \( \mu \)l/min, is high compared to most tissues, is five times that of the brain as a whole (20), and is above the highest measured for any nuclear mass (23).

**Validity of change in hematocrit as a measure of relative loss of volume from plasma compartment.** It is necessary to examine two questions—the equivalence of the aortic and the choroidal arterial hematocrits, and the equivalence of average erythrocyte volume in arterial and venous blood of the choroid plexus.

With respect to the first, it has not been possible to sample arterial blood from branches of the posterior choroidal artery entering the plexus. The issue is whether there is an unequal distribution of cells and plasma in the cerebral circulation. Measurements of the hematocrit of blood from the cortical surface have shown a slightly higher hematocrit than that of blood from the aorta and this suggests that there may be some slight cell separation in the distribution of blood to the brain. If there be such an unequal distribution, its effect upon the blood of the choroid plexus cannot be predicted. Moreover, any difference which might exist should be considerably reduced by the removal of the superior portions of both cerebral hemispheres for the exposure of the plexus. That the hematocrit of the venous blood approaches equality with that of the aorta after the administration of acetazolamide certainly shows that large or significant differences between aortic and choroidal arterial blood do not exist.

The second question refers to the maintenance of a constant average volume of erythrocytes during passage of blood through the plexus. Swelling of erythrocytes associated with increased \( CO_2 \) content of venous blood should not introduce a significant change. Jackson and Nutt (19) measured this effect in erythrocytes of several species, including the rabbit, and found that, although erythrocytes of the rabbit swelled between 8-13% with change of tension of \( CO_2 \) from 5 to 700 mm Hg, it was impossible to measure a significant change over the physiological range of values.

Adventitious osmotic shifts of water from plasma to ventricle or in the reverse direction would introduce serious change in erythrocyte volume. Erythrocytes of the rabbit in heparinized plasma might be expected to behave as almost perfect osmometers as do erythrocytes of human subjects (15). Thus, desiccation or dilution of the plasma would be accompanied by change in volume of erythrocytes which would tend to restore the original hematocrit. Relatively large movement of water would be required to produce a small change in hematocrit. Therefore, changes in average volume of erythrocytes occasioned by a shift of water between plasma and ventricle would tend to go unnoticed, and in this sense the difference in hematocrit between the arterial and venous blood is a reasonably imperturbable measure of movement of solute. In this connection it is worthy of note that under the circumstances of these experiments, the greater part of the choroid plexus was bathed in cerebrospinal fluid which was being continuously renewed and there is, in the analyses made by de Rougemont et al. (9), evidence that nascent fluid is not very different from plasma in total osmolarity. Finally, if due allowance is made for the inaccuracy of the erythrocyte counts, these have not, when compared with the hematocrits, revealed large changes in average volume of erythrocytes.

**Secretion of cerebrospinal fluid.** Several estimates have been made of the rate of formation of cerebrospinal fluid in the rabbit and these, together with data from other species, are summarized in Table 3. The amount of fluid formed by the plexus is in the range of that measured for cerebrospinal fluid as a whole. The data, however, give no assurance that an increment of fluid does not arise from the nervous tissue nor yet that a fraction, secreted by the plexus, might not be lost to the brain. Under the influence of acetazolamide the position with respect to a dual source is clear and this will be returned to later.

First, however, comment ought to be made about the maximum rate of formation of fluid which has been measured. In several instances this has come to an amount of fluid each minute almost equal to the weight...
of the plexus, a performance which rivals the highest rates of reabsorption of glomerular filtrate by the kidney.

**Effect of acetazolamide.** Acetazolamide in a dosage of 150 mg/kg was found by Tschirgi, Frost, and Taylor (33) to reduce the open outflow of cerebrospinal fluid by some 70% in cats and rabbits and, in the undrained system, to reduce the pressure of cerebrospinal fluid by some 50%. This result was confirmed by Kister (21), who found the effect independent of dosage above .5 mg/kg and, in respect to the pressure of the fluid, by Knopp, Atkinson, and Ward (22) in cats but not in normal monkeys; by Elvidge, Branch, and Thompson (11) and Birzis, Carter, and Maren (9) for hydrocephalic children, and the latter also for nonhydrocephalic human subjects.

The rate of turnover of radioactive sodium in cerebrospinal fluid was found to be reduced by acetazolamide in experiments by Davson and Luck (8) in several species, including the rabbit, and by Fishman (12) in dogs, and the rate of renewal of cerebrospinal fluid, as estimated from the rate of disappearance of intrathecally injected radioactive iodinated albumin in dogs, was found by Van Wart, Dupont, and Krainitz (34) to be reduced to about half by the administration of 25 mg/kg acetazolamide.

By the more precise method of ventriculocisternal perfusion, Pollay and Davson (29) found a reduction of production of cerebrospinal fluid of approximately 50% in rabbits after the administration of 100 mg/kg acetazolamide or during the ventricular perfusion of a solution containing .2 mg/ml.

In addition to a 40% reduction in the rate of turnover of Na+, Davson and Luck (8) found a change in the anionic composition of the fluid after the administration of acetazolamide, the excess of Cl− being reduced and the anionic pattern thus approaching that expected in a dialysate of plasma. The reduction in concentration of Cl− which was also found by Marcu and Fischer (24) in elasmobranchs, and Maren and Robinson (25) in human fluid, led Davson and Luck (8) to predict that the rate of formation of choroidal fluid was depressed to a greater extent than that measured by them for fluid as a whole, the difference being made up by the entrance into the cerebrospinal fluid compartment of fluid from nervous tissue.

Even with these considerations in mind, it was surprising to find, in the initial experiments carried out in an uncontrolled environment, instead of a decrease of 50 or 70% in production of fluid by the plexus, a complete or nearly complete cessation of secretion. The possibility existed that, in the intact animal, the uncatalyzed hydration of CO2 provided carbonic acid at a rate sufficient to maintain 30–50% of the normal secretion of fluid by the plexus, but that in the preparation employed, the escape of CO2 into the atmosphere caused the provision of carbonic acid to be reduced to a rate insufficient to sustain significant secretion. When the experiments were carried out under circumstances calculated to prevent the loss of CO2 or to increase its concentration in the fluid bathing the plexus, the severity of inhibition was not attenuated. To account, therefore, for the difference between the effect of acetazolamide upon the formation of fluid in general and that from the choroid plexus, it seems necessary to conclude, in agreement with the early prediction of Davson and Luck (8), that under the influence of acetazolamide there are, indeed, dual sources of fluid and, in terms of sensitivity to the inhibitor, dual mechanisms of formation as well.

**Ion-exchange hypothesis.** On the basis of their observation that acetazolamide results in a decrease in the rate of external drainage of cerebrospinal fluid, Tschirgi et al. (33) proposed that the fluid might be formed by an ion exchange between plasma and fluid, Na+ and Cl− with osmotically obligated water from the plasma exchanging with H+ and HCO3− derived from the hydration of metabolically produced CO2. As conceived and later elaborated (32), the proposal envisioned the formation of fluid to occur from capillaries generally throughout the central nervous system, including the capillaries of the choroid plexus. That, under the influence of acetazolamide, the rate of turnover of Na24 in the brain was found by Woodbury et al. (36) to be reduced was cited in support for the surmise. The cited reduction in turnover of sodium was small, however, in relation to that which might be required, and Davson and Luck (8) had found no change in rate of turnover of Na24 in brain after administration of the drug. This still left the choroid plexus, however, as a possible site for the operation of an ion exchange of the type outlined, although the availability to the plexus of the quantities of CO2 which would be required seems unlikely.

If such a mechanism were operating, the consequences, in terms of the content of CO2 in the venous blood from the plexus, would be curious. At the rates of formation of fluid which have been measured for the plexus, approximately 24 mEq of sodium ions are secreted, on an average, per liter of blood leaving the plexus, and an equivalent amount of CO2 would be expected to be added to the blood were the ion-exchange hypothesis correct. Measurements of the CO2 content of arterial
blood and venous blood from the plexus have not shown
increases of the order necessitated (Table 2).

Effect of ouabain. Since the demonstration by Schatz-
mann (31) of the inhibitory action of cardiac glycosides
upon cation transport by erythrocytes, these agents have
been found to interfere with the active transport of a
number of substrates in several tissues. The action on
transport has been associated by Post et al. (30) and by

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