Hydrocephalus: Changes in Formation and Absorption of Cerebrospinal Fluid Within the Cerebral Ventricles*

EDGAR A. BERING, JR., M.D.† AND OSAMU SATO, M.D.

Neurosurgical Research Laboratory, Children's Hospital Medical Center, and Department of Surgery, Harvard Medical School, Boston, Massachusetts

This paper presents a study designed to measure accurately the changes that occur in the formation and absorption of cerebrospinal fluid within the cerebral ventricles during the development of hydrocephalus. The results of these experiments are considered in relation to the evaluation of the hydrocephalic patient.

A hydrocephalic infant on ventricular drainage will produce several hundred cc. of cerebrospinal fluid per day, but the ventricular rate of growth in rapidly progressing hydrocephalus is only of the order of 10 to 15 cc. a day, only a small fraction of the amount of fluid being formed. Clearly, therefore, there must be intraventricular absorption of fluid. Attempts have been made to measure absorption of cerebrospinal fluid for the evaluation of hydrocephalus by measuring the disappearance of tracers from the fluid, but this method has been shown to be wrong fundamentally.2 The data required to evaluate the outflow of cerebrospinal fluid or absorptive mechanism must include information about the rate of formation of the bulk of the fluid and how it can change. What is the maximum production of cerebrospinal fluid that the system of outflow must handle? There also must be information about the resistance to outflow of the bulk of cerebrospinal fluid (or absorption) and the total absorptive capacity. With these data an accurate assessment can be made of the absorptive mechanism and its ability to handle the cerebrospinal fluid produced.

Recently, methods have been developed for measuring accurately the formation and absorption of cerebrospinal fluid.16 These have been applied to a large group of normal dogs and dogs with acute and chronic progressive hydrocephalus. This provided data about the physiology of cerebrospinal fluid, and an opportunity to test this type of data for the evaluation of hydrocephalus.

Material and Methods

These studies were done on male mongrel dogs weighing 12 to 17 kg. anesthetized with intravenous pentobarbital. The animals were prone with the head slightly elevated so that the external auditory meatus was 20 cm. above the top of the table. All procedures were carried out aseptically.

The experimental data were obtained from perfusion of the ventricular system with artificial cerebrospinal fluid by the method described in detail by Pappenheimer et al.11,14 but with some modifications to adapt it to dogs. Ventricular punctures were made percutaneously through needle guides imbedded previously in the skull and the scalp was closed over them. Cisternal punctures were made percutaneously through the foramen magnum. The rates of perfusion were in the range of 0.23 to 0.33 ml./min., but constant for any one experiment to ±0.002 ml./min. Intra-ventricular pressure was measured continuously at the inflow needle with a Statham strain gauge feeding into a Grass polygraph. Other physiological events (electrocardiograms, electroencephalograms and arterial and venous blood pressures) also were monitored on the polygraph. The system was tested for leaks at the time of sacrifice by perfusion with a mixture of 10 per cent formalin, isotonic saline and methylene blue. Then the calvarium was removed and the brain was inspected for leaks or the animal was decapitated and the head was frozen and cut in the frozen state. The presence of dye outside the desired area was considered to constitute a leak and the

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† Present address: National Institute of Neurological Diseases and Blindness, Bethesda 14, Maryland.
experiment was discarded. Other animals were sacrificed with intravenous Nembutal, and the brain was removed and fixed in formalin or other appropriate fixatives for anatomical studies.

The perfusive fluid was made up to the average composition of cerebrospinal fluid of the dog found in this laboratory\(^4\) and adjusted for pH and content of CO\(_2\) as described.\(^16\) Various tracers were added in small amounts which did not affect its osmotic concentration.

Determinations of creatinine were done by the alkaline picrate method; inulin by the resorcinol method; albumin tagged with T\(^{38}\) (RISA) was counted in a well-scintillation counter; and the C\(^{14}\) urea was counted in a liquid-scintillation or a continuous gas-flow counter. The rates of inflow and outflow were measured gravimetrically to ± .002 gm. and ± .00002 gm. per min.

The interpretation of the results of these experiments involved small difference between relatively large quantities so that accuracy of all measurements was extremely important and considerable effort was made to reduce all errors to less than 2 per cent both by attention to technique and by multiple determinations.

The notation to be used in the description of the results will follow that used by Heisey et al.\(^11\)

\[
\dot{V} = \text{rate of flow mL/min.}
\]
\[
i, o, p = \text{subscripts referring to inflow, outflow, and plasma respectively.}
\]
\[
f, a = \text{subscripts referring to formation and bulk absorption of fluid.}
\]
\[
c = \text{concentration of quantity/ml.}
\]
\[
\bar{c} = \text{mean concentration in ventricular system} = \bar{c}_o + 0.37 (c_i - c_o)
\]
\[
\dot{n}_x = \text{steady-state transport of any substance (x) from the perfusion of cerebrospinal fluid to the blood = V} \dot{c}_i - V \dot{c}_o
\]
\[
C_x = \text{steady-state clearance of x = } \dot{n}_x / \bar{c}.
\]
\[
c_o = \text{can be used as well as the } \bar{c}.
\]

This gives a slightly higher figure and is correct for the subarachnoid space.

Four types of perfusion were done in the normal dogs: subarachnoid-cisternal perfusion, ventriculo-cisternal perfusion, ventriculo-aquaduct perfusion, and lateral ventricle-lateral ventricle perfusion with the aqueduct of Sylvius blocked.

There were three groups of perfusions in hydrocephalic dogs. Lateral ventricle-4th ventricle and lateral ventricle-lateral ventricle perfusions in dogs made hydrocephalic by cisternal kaolin,\(^21\) and lateral-ventricle to lateral-ventricle perfusion in dogs made hydrocephalic by plugging the aqueduct of Sylvius.\(^12\)

During perfusion, the pressure of perfusion was regulated by the height of the outflow, where a proportional drop counter recorded the outflow. Perfusion was carried on at any one pressure for 45 min., then 15-min. samples were taken until two successive samples had rates of inflow and outflow constant within 2 per cent. When the tracers were first added the 45-min. period was broken up into three 15-min. intervals so that the volume of distribution could be calculated. Several pressures were used in any one experiment, usually at increments of 100 mm. H\(_2\)O. The resistance of the inflow needle was determined separately for each experiment and the appropriate correction in pressure was made.

**Results**

**Volumes of Distribution.** The ventricular volume was measured by calculating the volumes of distribution of the various tracers used. The calculation of the volume of distribution of any substance depended upon the amount of the substance remaining in the cerebrospinal fluid after a steady state was reached, taking into account the amount absorbed from the fluid and the amount in the dead space of the system of perfusion. The following formula was used for this calculation.\(^16\)

\[
VD_x = \frac{\sum c_i [\dot{V}_i - \dot{V}_o c_0(t) - C_x \dot{c}_o(t)] \Delta t}{\dot{c}}
\]

where:

\[
VD_x = \text{volume of distribution of the test substance x.}
\]
\[
n = \text{number of samples.}
\]
\[
i, o = \text{subscripts referring to inflow and outflow.}
\]
\[
c_o(t), \dot{c}_o(t) = \text{the concentrations at time } t.
\]
\[
e_u(t) = \text{steady-state concentration of outflow.}
\]
\[
\dot{V}IT = \text{volume of inflow tubes.}
\]
\[
\dot{V}OT = \text{volume of outflow tubes.}
\]
\[
C_x = \text{the steady-state flux or clearance in mL/min. of x out of the ventricular system per unit concentration} = (\dot{V}_i c_i - \dot{V}_o c_o) / \dot{c}
\]

In the normal dog, the volume of distribution of subarachnoid (parietal)-cisterna magna perfusion was 7.9 ml. for creatinine and 5.8 ml. for inulin. The mean volumes of distribution found in ventriculocisternal
perfu-
sions which included the lateral, 3rd and 4th ventricles, the subarachnoid space of the posterior fossa, and the cisterna magna were 3.9 ml. for creatinine, 3.0 ml. for inulin, 5.2 ml. for RISA, and 7.6 ml. for urea.

The combined volumes of the two normal lateral ventricles and the 3rd ventricle obtained from 11 ventricular-ventricular perfusions with a blocked aqueduct of Sylvius were 1.8 ml., 1.6 ml., 2.2 ml., and 2.4 ml. for creatinine, inulin, RISA, and urea. The 4th ventricle had a volume of .2 ml. as measured by planimetry of serially sectioned formalin-fixed brains. This indicated that the subarachnoid space of the posterior fossa had a volume of about 2 to 2.5 ml. which was in good agreement with approximate values obtained from the difference between the volumes of the posterior fossa, and the cerebellum and medulla measured post mortem. This method gave volumes of 1.6 to 3 ml. for the posterior fossa, subarachnoid space and cisterna magna depending on the size of the dog.

The mean total weight of the brain of dogs of this size was 76 (±1.6) gm. with a total weight of choroid plexus of 79 (±7) mg. The choroid plexus of each lateral ventricle weighed 20 (±2.4) mg. and that in the 4th ventricle, 39 (±4.0) mg.

Hydrocephalic dogs (mean of 55 days in duration after cisternal kaolin) showed larger volumes of distribution, as expected. The mean values from 16 experiments were 5.5 ml., 3.7 ml., and 4.2 ml. for creatinine, inulin, and RISA. These volumes included only the ventricular system as the kaolin caused blockage of the foramina of Luschka and indicated about a threefold enlargement of the ventricular system in this period of time.

Hydrocephalus produced by plugging the aqueduct of Sylvius caused similar enlargement (Table 1).

The ventricular enlargement in kaolin hydrocephalus was progressive as shown by successive determinations over a period of 270 days (Fig. 1).

The small variations in the volumes of distribution were partly caused by analytical errors and partly by differences in characteristics of permeability of the tracers.

**Formation of Cerebrospinal Fluid.** Formation of cerebrospinal fluid was calculated by the method of Heisey et al.11 which combined the effects of hydrostatic pressure on outflow-inflow differences and on clearance of inulin. Outflow-inflow differences involved both formation and absorption of cerebrospinal fluid while clearance of inulin measured only bulk absorption. By combining

### TABLE 1

**Volumes of distribution***

<table>
<thead>
<tr>
<th></th>
<th>Creatinine</th>
<th>Inulin</th>
<th>RISA</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subarachnoid to cisterna magna</td>
<td>1 7.9</td>
<td>1 5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral ventricle to cisterna magna</td>
<td>11 3.9 (±0.5)</td>
<td>12 3.0 (±0.6)</td>
<td>3 5.2</td>
<td>4 7.6</td>
</tr>
<tr>
<td>Lateral ventricle to lateral ventricle with aqueduct of Sylvius plugged</td>
<td>10 1.8 (±0.3)</td>
<td>11 1.6 (±0.3)</td>
<td>3 2.2</td>
<td>8 2.4 (±0.5)</td>
</tr>
<tr>
<td>Hydrocephalic dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaolin hydrocephalus (mean 55 days in duration)</td>
<td>14 5.5 (±4.4)</td>
<td>16 4.7 (±0.6)</td>
<td>8 4.2</td>
<td>3 7.5</td>
</tr>
<tr>
<td>Aqueduct plug (50 days)</td>
<td>3 6.6</td>
<td>3 7.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean volumes of distribution of creatinine, inulin, RISA, and urea calculated from various experiments on perfusion in normal and hydrocephalic mongrel dogs (15 to 18 kg.). Values in parentheses are the standard errors of the mean.
 Hydrocephalus: Changes in CSF in Ventrices

Fig. 1. Changes in ventricular volume, slope of line of regression of effect of hydrostatic pressure on clearance of inulin (C.IN) which is a measurement of resistance to outflow of cerebrospinal fluid, and changes in gross morphology of ependymal cell which occur during development of hydrocephalus produced by injection of kaolin in the cisterna magna of the dog.

these data an expression for the rate of formation as a function of hydrostatic pressure was obtained.

The hydrostatic pressure of the system of perfusion controlled the outflow of cerebrospinal fluid. When the pressure was decreased the outflow increased and when the pressure was increased the outflow decreased. The outflow-inflow differences (O-I) were examined using two pressures. One was the pressure of cerebrospinal fluid, measured at the inflow, and the other was the differences between the pressure of cerebrospinal fluid and the venous pressure of the superior sagittal sinus. The lines of regression given in Table 2 were calculated as the least square lines assuming error in both x and y.26

When considering the effects of pressure of cerebrospinal fluid on the outflow-inflow difference, zero pressure for any one experiment was taken to be that pressure when outflow and inflow were equal. This gave the required data about the effect of changes in the hydrostatic pressure on outflow-inflow difference, and reduced scatter of data caused by the uncertainties of trying to select the same zero point in all animals. A linear relationship between pressure and outflow-inflow difference was found in the normal dogs over a range from −100 to +400 mm. H2O. Below −100 there was a sharp break in the line and any further decrease of pressure had little or no effect. This was similar to the maximum flow found when cerebrospinal fluid was allowed to drain freely at various pressures from the cisterna magna.3

The effective hydrostatic pressure in an animal was the difference between the pressure of the cerebrospinal fluid and the venous pressure. This plus the fact that the zero point of the cerebrospinal-fluid system seemed uncertain, led to the conclusion that the difference between the venous pressure of the superior sagittal sinus and the pressure of the cerebrospinal fluid was a more precise measure of the effect of pressure. It has been said that the venous pressure of the superior sagittal sinus is not affected by changes in the pressure of the cerebrospinal fluid,23 but in these experiments there were some changes in the venous pressure when the pressure of the cerebrospinal fluid was changed. These changes were not as marked in the normal dog as they were in the hydrocephalic dog. The outflow-inflow differences had a linear relationship to the cerebrospinal fluid-sagittal sinus pressure with a slightly steeper slope to the line of regression than when the pressure of the cerebrospinal fluid was used but not significantly so. This was true in both the normal dogs and the hydrocephalic dogs (Fig. 2 and Table 2).

There was no demonstrable connection between the subarachnoid space and the
ventricular system of the hydrocephalic dogs, and when the outflow was less than the inflow it meant that there was absorption of bulk of fluid within the cerebral ventricles. Many of the dogs that had had kaolin injected into the cisterna magna were found to have an enlarged central canal of the spinal cord. It was thought that this might possibly allow some leak to the subarachnoid space, but four similar experiments carried out in dogs made hydrocephalic by plugging the aqueduct of Sylvius also demonstrated absorption of cerebrospinal fluid in the cerebral ventricles.

When the outflow was equal to the inflow, the rate of absorption was equal to the rate of formation of cerebrospinal fluid, but if the outflow was greater than inflow, formation was greater than absorption. If the outflow were less than inflow more fluid was absorbed than was formed. This relationship of outflow-inflow difference to formation and absorption of cerebrospinal fluid can be expressed as:

$$\dot{V}_i - \dot{V}_o = \dot{V}_f - \dot{V}_a$$ (2)

The clearance of inulin (C_IN) calculated as described was found to vary linearly with hydrostatic pressure, in both the normal and hydrocephalic dog (Figs. 3 and 5, and Table

**TABLE 2**

<table>
<thead>
<tr>
<th>Normal dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venticulocisternal perfusion</td>
</tr>
<tr>
<td>(O-I) = 0.031 (±0.006) - 0.499 (±0.077) X 10^-3 (CSFP-SSVP)</td>
</tr>
<tr>
<td>(O-I) = -0.440 (±0.087) X 10^-3 CSFP</td>
</tr>
<tr>
<td>C_N = 0.017 (±0.006) + 0.438 (±0.089) X 10^-3 (CSFP-SSVP)</td>
</tr>
<tr>
<td>C_N = 0.047 (±0.008) + 0.447 (±0.078) X 10^-3 CSFP</td>
</tr>
<tr>
<td>C_H_F = 0.049 (±0.015) + 0.329 (±0.068) X 10^-3 (CSFP-SSVP)</td>
</tr>
<tr>
<td>C_H_F = 0.087 (±0.041) + 0.466 (±0.002) X 10^-3 (CSFP-SSVP)</td>
</tr>
<tr>
<td>C_R = 0.027 (±0.009) + 0.303 (±0.007) X 10^-4 (CSFP-SSVP)</td>
</tr>
<tr>
<td>C_R = 0.032 (±0.007) + 0.248 (±0.030) X 10^-3 CSFP</td>
</tr>
<tr>
<td>C_T = 0.048 (±0.008) - 0.031 (±0.032) X 10^-3 (CSFP-SSVP)</td>
</tr>
</tbody>
</table>

| Lateral ventricle-lateral ventricle perfusion |
| (O-I) = -0.16 X 10^-3 (±0.077) CSFP |
| C_N = 0.016 (±0.007) + 0.141 (±0.067) X 10^-3 CSFP |
| C_H_F = 0.031 (±0.009) + 0.160 (±0.050) X 10^-3 CSFP |
| C_R = 0.073 (±0.007) + 0.248 (±0.030) X 10^-3 CSFP |

| Hydrocephalic dog (kaolin) (55 days) |
| Venticular-4th ventricle perfusion |
| (O-I) = 0.024 (±0.008) - 0.404 (±0.059) X 10^-3 (CSFP-SSVP) |
| (O-I) = -0.391 (±0.054) X 10^-3 CSFP |
| C_N = 0.003 (±0.006) + 0.354 (±0.056) X 10^-3 (CSFP-SSVP) |
| C_N = 0.027 (±0.003) + 0.302 (±0.043) X 10^-3 CSFP |
| C_H_F = 0.037 (±0.009) + 0.303 (±0.007) X 10^-4 (CSFP-SSVP) |
| C_H_F = 0.017 + 0.296 (±0.030) X 10^-3 CSFP |
| C_T = 0.027 - 0.050 X 10^-3 (CSFP-SSVP) |

| Lateral ventricle-lateral ventricle perfusion (21 days) |
| C_N = 0.016 (±0.003) + 0.161 (±0.087) X 10^-3 CSFP |
| C_H_F = 0.060 (±0.003) + 0.131 (±0.082) X 10^-3 CSFP |
| C_R = 0.120 (±0.026) + 0.273 (±0.139) X 10^-4 CSFP |

* Various experiments on perfusion were performed in normal and hydrocephalic dogs. O-I = outflow-inflow difference; C_N = clearance of inulin; C_H_F = clearance of urea; C_R = clearance of creatinine; CSFP = cerebrospinal fluid pressure; SSVP = venous pressure of sagittal sinus. CSFP zero was taken to be the pressure when outflow-inflow were equal.
and was taken as a measure of absorption of bulk. The use of clearance of inulin as a measure of absorption of bulk was based on the assumption that diffusive absorption of inulin was sufficiently slow as to be negligible. When the pressure was low enough, the clearance of inulin went to zero and all the inulin put in came out (Fig. 3), which supported the assumption. The slope of the line was not significantly different in magnitude than that of the line of regression of the effect of pressure on outflow-inflow difference which again suggested absorption of bulk.

Therefore, the relation between clearance of inulin and absorption can be written:

\[
\dot{V}_a = C_{IN}
\] (3)

Substituting \( \dot{V}_f - \dot{V}_a \) for O-I and \( \dot{V}_a \) for \( C_{IN} \) in the appropriate equations of regression from Table 2 and combining them to eliminate \( \dot{V}_a \), an expression for \( \dot{V}_f \) was obtained:

For the normal dog (ventriculocisternal perfusion):

\[
\dot{V}_f = 0.0048(\pm.008) - 0.051(\pm.113) \times 10^{-3}
\] (4)

(pressure of cerebrospinal fluid-venous pressure of sagittal sinus)

For the dog with chronic kaolin induced hydrocephalus:

\[
\dot{V}_f = 0.027(\pm.010) - 0.050(\pm.081) \times 10^{-3}
\] (5)

(pressure of cerebrospinal fluid-venous pressure of sagittal sinus)

This is also shown graphically in Figs. 4A and 4B.

Although there appears to be a slight negative slope to the \( \dot{V}_f \) lines, they were not significantly different from zero. This means that, within the physiological range, pressure had no effect on formation of cerebrospinal fluid either in the normal or hydrocephalic state.

The normal rate of formation was taken to be that found at the pressure at which the outflow minus inflow was zero: where absorption and formation were equal.

Table 3 shows the rates of formation of cerebrospinal fluid found in the normal dog with the subarachnoid space open, in the normal ventricles with the aqueduct of Sylvius blocked, and in two types of hydrocephalic dogs. The rate of formation of the
fluid was found to be the same (.016 ml./min.) in the normal ventricle and in the hydrocephalic ventricle with the aqueduct of Sylvius occluded. This suggested that the decreased formation of cerebrospinal fluid seen in the chronic kaolin hydrocephalic dogs was the result of the exclusion of cerebrospinal fluid formed in the subarachnoid space and not an effect of the hydrocephalic process.

The distribution of the formation of cerebrospinal fluid in a 15-kg. dog would appear to be:

<table>
<thead>
<tr>
<th>Ventricle System</th>
<th>Rate (ml./min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular system</td>
<td>.027</td>
</tr>
<tr>
<td>Lateral and 3rd ventricles</td>
<td>.016</td>
</tr>
<tr>
<td>4th ventricle</td>
<td>.011</td>
</tr>
<tr>
<td>Subarachnoid space</td>
<td>.020</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>.047</strong></td>
</tr>
</tbody>
</table>

Table 3 also includes data obtained by free drainage of cerebrospinal fluid from normal and hydrocephalic dogs. The agreement was very good and suggests that the drainage as done was a true measure of the maximum production of cerebrospinal fluid.

**Absorption of Cerebrospinal Fluid.** Clearance of inulin was used as a measure of absorption of cerebrospinal fluid for reasons that have been given previously. Absorption, in contrast to formation of cerebrospinal fluid, was found to be linearly and directly related to hydrostatic pressure in both the normal and the hydrocephalic dog. Fig. 5 shows the calculated lines of regression for 33 points of clearance obtained from ventriculocisternal perfusions in the normal dog (solid line) and for 41 points of clearance from ventricular-4th ventricle perfusions in dogs with chronic kaolin hydrocephalus (broken line).

The clearance of inulin in the normal dog shows that there was some absorption at zero difference in pressure of cerebrospinal fluid and venous pressure of sagittal sinus and clearance of inulin did not go to zero until some pressure below this. This suggested that there was some other route for absorption of bulk of fluid other than into the venous system. Such a pathway has been dem-

---

**Fig. 4.** Formation of cerebrospinal fluid as measured by combination of outflow-inflow differences, and clearance of inulin as a function of hydrostatic pressure. (Left) Data from ventriculocisternal perfusion in normal dog. (Right) Data from lateral ventricle-4th ventricle perfusions in dogs with chronic kaolin hydrocephalus.
TABLE 3
Mean rate of formation of CSF*

<table>
<thead>
<tr>
<th></th>
<th>Rate of CSF Formation</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventriculocisternal</td>
<td>0.047 (± 0.006)</td>
<td>17</td>
</tr>
<tr>
<td>(Ventricular system and subarachnoid space)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular-ventricular</td>
<td>0.016 (± 0.007)</td>
<td>11</td>
</tr>
<tr>
<td>(acutely plugged aqueduct of Sylvius, lateral and 3rd ventricles only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drainage—from cisterna magna</td>
<td>0.046 (± 0.002)</td>
<td>46</td>
</tr>
<tr>
<td>(ventricular system and subarachnoid space)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocephalic dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaolin hydrocephalus</td>
<td>0.027 (± 0.006)</td>
<td>15</td>
</tr>
<tr>
<td>(lateral ventricle to 4th ventricle)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic aqueduct plug</td>
<td>0.016</td>
<td>4</td>
</tr>
<tr>
<td>(lateral ventricle to lateral ventricle)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drainage—from ventricle</td>
<td>0.030 (± 0.002)</td>
<td>15</td>
</tr>
<tr>
<td>(chronic kaolin hydrocephalus)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Dogs were under light pentobarbital anesthesia. Rate was measured by perfusion-clearance of inulin method and by free drainage. Figures in parentheses are the standard errors of the means.

onstrated in the dog through the cribiform plate into the tissues of the nose. Absorption in the hydrocephalic animal in which ventricles were isolated from the subarachnoid spaces went to zero at zero difference in pressure of cerebrospinal fluid and venous pressure of sagittal sinus which indicated that the movement of bulk of the cerebrospinal fluid out of the isolated cerebral ventricles was only into the venous system and dependent upon the difference between pressure of cerebrospinal fluid and venous pressure.

The slope of the lines of regression of clearance of inulin (Table 2) was the reciprocal of the resistance to flow or hydrostatic conductance which means that the steeper the slope, the less resistance to flow or the easier the absorption of fluid. The slopes of the two lines, one from the normal animal and one from the hydrocephalic animal, were not different statistically from each other which means that resistance to absorption of cerebrospinal fluid was not greater in the chronic progressive hydrocephalic animal than in the normal animal. These data were obtained from chronically hydrocephalic animals, and, as it is well known that the ependyma becomes flattened, stretched and, in some places, discontinuous as hydrocephalus develops, it was important to find out whether or not the ability to absorb fluid was the result of these ependymal changes or whether it also occurred in the normal ventricle. The dog produces about 25 ml. of cerebrospinal fluid in the ventricles per day and as the ventricles enlarge only 4 to 5 ml. in several weeks, any change must occur quickly.

This question was answered by a series of 7 acute experiments in normal animals in which the aqueduct of Sylvius was plugged with cotton and sealed with Eastman #910 Adhesive and a perfusion from one lateral ventricle to the other was carried out, and in another 6 animals in which the aqueduct of Sylvius was catheterized and a ventriculolateral perfusion was carried out. These experiments demonstrated conclusively that cerebrospinal fluid was absorbed in the normal ventricle. However, the resistance to absorption was greater than in the dog with the intact subarachnoid space, or in the ventricles of the chronic hydrocephalic dog. The slope of the line of regression was only 0.141 (± 0.067) × 10⁻³ ml. cerebrospinal fluid/min./mm. H₂O as compared to
TABLE 4
Coefficients of permeability*

<table>
<thead>
<tr>
<th></th>
<th>K_Dx (cm$^3$ min.$^{-1}$)</th>
<th>Urea (cm$^3$ min.$^{-1}$)</th>
<th>K_Dx (cm$^3$ min.$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dog</td>
<td>0.042</td>
<td>0.77</td>
<td>0.133</td>
</tr>
<tr>
<td>Ventriculocisternal perfusion (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal dog</td>
<td>0.017</td>
<td>0.61</td>
<td>0.048</td>
</tr>
<tr>
<td>Isolated lateral ventricles (10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocephalic dog</td>
<td>0.047</td>
<td>1.23</td>
<td>0.075</td>
</tr>
</tbody>
</table>

* Calculated coefficients of diffusive permeability of creatinine and urea. The mean pore radius was calculated from the ratio of those two. Figures in parentheses are the number of experiments.

0.447(±0.078)$\times10^{-3}$ ml. cerebrospinal fluid/min./mm. H$_2$O when the subarachnoid space was intact, and 0.302(±0.043)$\times10^{-3}$ ml./cerebrospinal fluid/min./mm. H$_2$O in the chronic kaolin hydrocephalic dog.

A series of experiments done serially as hydrocephalus developed showed the slope of the clearance of inulin to increase (decrease in resistance to absorption) with time as the ventricular volume increased and the ependyma became flattened (Fig. 1).

The amount of cerebrospinal fluid which could be absorbed in the normal ventricle under mean normal pressures of 102 mm. H$_2$O was calculated from these data to be 0.015 ml. per min. As only 0.016 ml. of cerebrospinal fluid was formed per min. in the normal ventricle, an increase in pressure of only 8 mm. H$_2$O was required for all the fluid to be absorbed. The reduced resistance to absorption which occurs as hydrocephalus develops eliminated even this requirement of small pressure.

Clearance of Other Substances. Clearance of creatinine, RISA, and C$^{14}$ urea were also measured simultaneously with inulin. Urea and creatinine are both inert lipid insoluble molecules smaller than inulin, and when the clearance of inulin was zero, there was always some clearance of these substances which was considered to be a measure of their diffusive absorption.

Using the equations given by Heisey et al.,

$$K_{Dx} = \frac{n_x - C_{IN}}{\bar{c} - cp}$$

(6)*

where

$$n_x = \text{total flux of } x \text{ out of cerebrospinal fluid}$$

$$cp = \text{concentration of } x \text{ in blood}$$

For substances not appearing in blood:

$$K_{Dx} = \frac{n_x - C_{IN}}{C_x - C_{IN}}$$

(7)*

The calculation of $K_{Dx}$ for creatinine had to take into account the fact that the level of creatinine in the serum of the dog had a mean value of 2 mg. per cent which was about 20

* Equations (6) and (7) are slightly different than those given by Heisey et al. They give these equations as:

$$K_{Dx} = \frac{n_x - C_{IN}c_0}{\bar{c} - cp}$$

for equation (6), and for equation (7):

$$K_{Dx} = C_x - C_{IN} \frac{c_0}{\bar{c}}$$

However, in calculating the data of these experiments, all clearances were calculated using $\bar{c}$ so the equations for the present calculations appear in the form as given in (6) and (7).
per cent of the concentration of creatinine in the perfusive fluid. Also when perfusing with creatinine-free fluid the outflow often contained as much as 0.5 to 1.0 mg. per cent creatinine. These values were measured in all experiments.

The \( K_{Dc} \) calculated for creatinine and urea in the various experimental situations is given in Table 4. These were used to calculate the mean pore radius in the ventricle by equations developed from the theory of restricted diffusion.\(^{11,16,17,19} \)

\[
\frac{K_{Dc}'}{K_{Dc}} = \frac{D_u}{D_e} \times \left( \frac{1 - \frac{ac}{r}}{1 - \frac{au}{r}} \right)^2 \frac{1}{1 - 2.10 \left( \frac{au}{r} \right)} + 2.09 \left( \frac{au}{r} \right)^3 - 0.95 \left( \frac{au}{r} \right)^5
\]

where:

- \( r = \) mean pore radius.
- \( K_{Dc}' = \) coefficient of diffusive permeability of urea.
- \( K_{Dc} = \) coefficient of diffusive permeability of creatinine.
- \( D_e, D_u = \) the free diffusive coefficients of creatinine and urea (1.2 \( \times \) 10\(^{-5} \) and 1.9 \( \times \) 10\(^{-5} \) cm.\(^2\) sec.\(^{-1}\)).
- \( ac, au = \) the Einstein-Stokes radii for creatinine and urea (3.4 \( \AA \)
- 2.6 \( \AA \))

substituting these values in equation (7) and the appropriate values for \( K_{Dc}' \) and \( K_{Dc} \) from Table 4, the mean pore radius \( r \) was calculated.

These calculations gave a mean pore radius of 8.5 for the ventricular system of the normal dog and the subarachnoid space of the posterior fossa which agree well with data from goats.\(^{11} \) The isolated normal ventricle had a larger mean radius of 10 \( \AA \) which increased to as much as 35 \( \AA \) in the chronic hydrocephalic animals. The radius of 10 \( \AA \) in the normal ventricle would seem to exclude the passage of inulin which has a radius of 15 \( \AA \), but this difference is within the error of measurement.

Data on permeability usually are expressed in terms of surface area and in order to allow comparison of these data with other biological investigations some estimates were made of the surface areas. This was done by perimetry of the cerebral ventricles and cerebellar surface carried out on serially sectioned formalin-fixed brains. All of these areas probably were low because of shrinkage during fixation. The cerebellar area was even more in error because only the visible surface of the cerebellum was included, which probably was much lower than the effective surface. The total surface of the cerebellum has been estimated as 1.6 times the visible area.\(^{7,13} \) The surfaces of the meninges were neglected completely which probably was a false assumption. The surface area of the choroid plexus was calculated from the results of Voetmann\(^{22} \) who estimated the choroid plexuses of man to have an area of 120 cm.\(^2\)/gm. of choroid plexus. The areas estimated for the normal and hydrocephalic dogs are given in Table 5.

The movement of albumin was studied using \( I^{131} \) tagged albumin (RISA). The clearance of RISA varied with pressure as did the other molecules, but it was always greater than clearance of inulin. This loss could not be by diffusion as it was a considerably larger molecule than inulin, and this must be explained either by entry into metabolic activity or into other local physical processes such as adsorption of surface. More experiments are required to settle this.

**Comment**

The constant rate of formation of cerebrospinal fluid independent of hydrostatic pressure found in these experiments confirms the work of others in unanesthetized animals\(^{11} \) and it probably is true for all the higher mammals. However, formation of cerebrospinal fluid can be affected by metabolic and other physiological changes, although there is
little detailed knowledge of these effects except for total changes in osmotic pressure of blood.\textsuperscript{11}

The relatively large amount of cerebrospinal fluid formed in the subarachnoid space was unexpected, but it is of some interest that this was about the same amount as was leaving by a route other than into the venous system. Whether or not this was coincidental or related in some way could not be said. The question of formation of subarachnoid cerebrospinal fluid and extravascular absorption of cerebrospinal fluid deserves more work and particular attention should be given to differences of species.

Assuming that all the cerebrospinal fluid produced within the cerebral ventricles came from the choroid plexuses, the rate of formation was 0.40 ml. cerebrospinal fluid/min./gm. of choroid plexus in the lateral ventricles while in the 4th ventricle it was only 0.275 ml. cerebrospinal fluid/min./gm. of choroid plexus. The choroid plexuses from the 4th ventricle of the chronic kaolin hydrocephalic were the same weights (40±8 mg.) as the normal (39±4 mg.) so that they would not seem to have been involved in the meningitis occluding the outlets of the 4th ventricle which might have caused a decrease in the output of cerebrospinal fluid. The situation was reversed if the entire ependymal area of the ventricular walls and choroid plexuses was assumed to produce cerebrospinal fluid. This gave a rate of formation of $0.6 \times 10^{-3}$ ml./min./cm.$^2$ of ependyma for the lateral ventricles, but in the 4th ventricle, it was twice this, $1.8 \times 10^{-3}$ ml./min./cm.$^2$ ependyma. If all the production of cerebrospinal fluid assigned to the subarachnoid space were considered to be coming from the 4th ventricle, the rates were still very different.

This apparent difference in rate of production of cerebrospinal fluid could be resolved if the cerebrospinal fluid was produced by both the ependyma and the choroid plexus and the rate for each tissue was the same in the lateral and 4th ventricles. This gave calculated rates of $0.24$ ml. cerebrospinal fluid/min./gm. of choroid plexus and $0.30 \times 10^{-3}$ ml./min./cm.$^2$ of ependyma of ventricular wall. Thus in the normal dog, about 67 per cent of the cerebrospinal fluid produced in the lateral ventricles came from the choroid plexuses and in the 4th ventricle about 93 per cent of the cerebrospinal fluid came from the choroid plexuses. The figure of $0.24$ ml. cerebrospinal fluid/min./gm. of choroid plexus agrees well with the figure of $0.20$ ml./min./gm. of choroid plexus found from drainage of cerebrospinal fluid after choroid plexectomy of the lateral ventricles.\textsuperscript{3} It was lower than the figure of $0.37$ ml./min./gm. of choroid plexus

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
 & Ventricular Wall & Choroid Plexus & Total \\
 & cm.$^2$ & cm.$^2$ & cm.$^2$ \\
\hline
Normal dog & & & \\
Lateral and 3rd ventricles & 21 & 5 & 26 \\
4th ventricle & 3 & 5 & 8 \\
& & 4 & 54 \\
Cerebellum and brain stem (visible surface only) & & 20 & \\
Total normal ventricular system and cerebellum and brain stem & & 54 & \\
Kaolin hydrocephalic dog (8 ml. vol.) & & & \\
Lateral and 3rd ventricles & 33 & 5 & 38 \\
4th ventricle & 6 & 5 & 11 \\
Total & & & 49 \\
\hline
\end{tabular}
\caption{Estimations of surface areas*}
\end{table}

* Estimations of the ependymal surface area of the cerebral ventricles and cerebellum of normal and kaolin hydrocephalic dogs were measured by planimetry on serial sections of formalin-fixed brains. Choroid-plexus areas were calculated from data of Voelmann.\textsuperscript{20}
calculated by Heisey et al.\textsuperscript{13} for the goat. However, they did not consider the possibility of either subarachnoidal formation of cerebrospinal fluid or cerebrospinal fluid coming from the ependyma of the ventricular wall and arbitrarily assigned all production of cerebrospinal fluid to the choroid plexuses.

The calculated uniform rates of production of cerebrospinal fluid would require blood flow of about 2 ml. blood/min./gm. of choroid plexus and .3 ml. blood/min./gm. of tissue for the subependymal blood flow if 20 per cent of water was removed from the plasma and the hematocrit was 40 per cent. These rates of blood flow and rates of extraction of water are reasonable in light of known physiological data.

The quantitative data on the absorption of bulk of cerebrospinal fluid within the normal and hydrocephalic ventricle are new but some confirmative evidence of intraventricular absorption of cerebrospinal fluid can be found in previous experimental work. Intraventricular absorption of cerebrospinal fluid was demonstrated by Nafigas\textsuperscript{14} and by Wislocki and Putnam\textsuperscript{21} in experiments on hydrocephalus. It was also demonstrated in experiments with hypertonic solutions\textsuperscript{10} but this was thought to be a special case. Flexner and Winters\textsuperscript{8,9} catheterized the aqueduct of cats and showed that the amount of fluid recovered was linearly related to the pressure of outflow. They interpreted this to mean that formation of cerebrospinal fluid was dependent on pressure, but now that the method of ventricular perfusion has demonstrated formation of cerebrospinal fluid to be independent of pressure, the experiments of Flexner and Winters can be said to demonstrate intraventricular absorption of cerebrospinal fluid linearly related to the hydrostatic pressure, as found in the present experiments.

It must be made clear that these data show only that intraventricular absorption of cerebrospinal fluid is possible, but they do not show what fraction of the fluid, if any, is absorbed by this route in the normal course of events. When the ventricles are separated from the subarachnoid space as in obstructive hydrocephalus, formation of cerebrospinal fluid continues unchanged and all the fluid must be absorbed within the ventricles.

These experiments do not provide all of the data required for precise evaluation of the circulation of cerebrospinal fluid as set forth in the introduction as there was no information obtained about variations of formation of cerebrospinal fluid or about the possibility of a maximum rate of absorption of cerebrospinal fluid. However, they were much more extensive than any data that have been available previously. Good data were obtained in 35 studies on normal dogs and 40 studies on hydrocephalic dogs so that there was a series of some 76 which could be examined as individuals. Using data on absorption alone, the progressive hydrocephalic could be recognized with reasonable certainty only in the very acute state, but the wide variation among the normals made even the detection of this group difficult which was not surprising in view of the change in resistance to absorption of cerebrospinal fluid which occurs as hydrocephalus progresses. The decreased rate of formation of cerebrospinal fluid found in the hydrocephalic animals was a much better clue to the clinical state than resistance to absorption, but it was not good enough to suggest its use as a clinical test.

The question immediately arises as to why the normal and the hydrocephalic cannot be distinguished by data of cerebrospinal-fluid flow and why ventricular enlargement occurs if the resistance to absorption of cerebrospinal fluid is not increased in hydrocephalus? The answer to this question lies mainly in the fact that the intraventricular pressure has always been considered as if it were constant, but this is not so; it varies enormously and continuously.\textsuperscript{1,4}

The cerebral ventricles will enlarge only if there is a difference in pressure between the cerebrospinal fluid and the brain. If the intraventricular pressure were constant, any change probably would be compensated by the movement of fluid into the brain equalizing the pressure, and unless the pressure were changed again, there would be no gradient of pressure and no ventricular enlargement.
However, the pressure is fluctuating continuously and it is this situation that must be considered. Blood filling the choroid plexus with each pulse increases the intraventricular contents and causes the intraventricular pressure to rise abruptly.\(^1\)\(^2\) The total time of rise usually is less than a second, and it is this sudden rise in intraventricular pressure or rate of increase in ventricular contents that is the key to the problem. The brain is inelastic and in a rigid box so that this change in volume must be countered either by loss of cerebrospinal fluid or venous blood. The formation and absorption of cerebrospinal fluid is too slow and too small in volume to do this so that it must be done by the venous system. The increasing pressure of the cerebrospinal fluid on the large veins of the cerebral hemispheres causes a momentary increase in the rate of venous outflow easing the pressure. As the choroid plexus empties there is a return of fluid toward the ventricles so that the cerebrospinal fluid has a to-and-fro motion which can be seen easily in Pantopaque cineventriculography. When the movement of cerebrospinal fluid through its normal pathways is blocked or venous outflow is impeded sufficiently,\(^4\) this cannot occur and the pressure of the pulse produced by each filling of the choroid plexus will increase, creating an increased cerebrospinal fluid-brain gradient of pressure with resulting ventricular enlargement.

Thus it becomes evident that ventricular enlargement is the result of the failure of the craniospinal contents to adjust to the rapidly fluctuating pressures caused by small but rapid changes in the intraventricular contents with each pulse of the choroid plexus. What is needed for the evaluation of hydrocephalus is exact knowledge of how this adjustment is accomplished, what variations the brain can withstand, and a method to measure it.

**Summary**

1. Formation, flow and absorption of cerebrospinal fluid were measured in normal and progressively hydrocephalic dogs by steady-state studies during ventricular per-

fusion. The formation of cerebrospinal fluid was found to be constant, independent of hydrostatic pressure, and unaffected by the development of hydrocephalus. The normal dog produced 0.016 ml. cerebrospinal fluid /min. in the lateral ventricles, 0.011 ml. cerebrospinal fluid/min. in the 4th ventricle, and 0.20 ml. cerebrospinal fluid/min. in the subarachnoid space. The intraventricular formation of fluid was calculated to be 0.94 ml. cerebrospinal fluid/min./gm. of choroid plexus, and 0.3\( \times 10^{-3} \) ml. cerebrospinal fluid /min./cm.\(^2\) of ependymal surface.

2. Absorption of cerebrospinal fluid was found to take place within the ventricles as well as in the subarachnoid spaces. The resistance to absorption of cerebrospinal fluid in the normal ventricle was much higher than in the hydrocephalic ventricle. The decrease in resistance occurred as the ventricular volume increased and the ependyma became flattened so that in chronic hydrocephalus the resistance was not significantly different than in the normal dog. The theory of restricted diffusion was applied to the data to calculate the diffusive permeabilities of creatinine and urea, and from this the mean pore radius within the ventricle.

3. Using formation of cerebrospinal fluid and data on absorption, the hydrocephalic animals could not be distinguished from the normal with sufficient accuracy to suggest using this as a clinical test. The reason for this is that intraventricular pressure has always been considered as if it were constant when, in fact, it is changing constantly. The problem of ventricular enlargement concerns the ability of the craniospinal contents to adjust to and absorb the small but very rapid changes in intracranial contents that occur with each beat of the pulse.

**References**

3. **Bering, E. A., Jr.** Problems of the dynamics of the cerebrospinal fluid with particular reference to