THE METABOLIC ACTIVITY OF THE CHOROID PLEXUS*

ROBERT G. FISHER, M.D., AND JOHN H. COPENHAVER, JR., PH.D.

Hitchcock Clinic, and Dartmouth College and Medical School, Hanover, New Hampshire

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The role of the choroid plexus in the production of cerebrospinal fluid is not clear. Even the most recent methods utilizing tracer techniques have provided neither complete understanding of the physiology of the fluid nor any clarity as to the mechanism of its formation.

Many have assumed that the choroid plexus is responsible for the production of cerebrospinal fluid. In 1664 Willis thought that the juxtaposition of the choroid plexus and pineal gland was related to the formation of this fluid. However, many years elapsed before the choroid plexus was actually studied scientifically. The Johns Hopkins group of Weed,28 Flexner,8 and Dandy6 made major advances in understanding the nature of the fluid and its flow in studies carried out in animals and man. These experiments indicated that the source of this fluid lay in the ventricles and that any obstruction to the flow of cerebrospinal fluid through the ventricular system caused increased intracranial pressure. Removal of the choroid plexus seemed to indicate that the pressure caused by obstruction could be relieved; the inference that the plexus must be responsible for the fluid was clear.

These studies dominated the thoughts about the flow of cerebrospinal fluid until the isotope studies of Sweet,24-26 Selverstone,26 Bakay,2 and Bering.3 These led to the hypothesis that all cerebrospinal fluid is not elaborated by the plexus but rather—"CSF is both a secretion and an ultrafiltrate. The choroid plexuses secrete it under a head of pressure sufficient to evoke ventricular dilatation and increased intracranial pressure if absorption is impeded. CSF also enters through the ependyma and the walls of the subarachnoid space as an ultra-filtrate. This method of formation depends upon the balance between the hydrostatic plus osmotic pressures in the blood on the one hand and CSF on the other, as well as on the permeability of the layer between them."24

On the other hand, Herlin,14 in a recent treatise on the metabolic barriers of the central nervous system, stated: "It is reasonable to presume that the choroid plexus strictly regulates the composition of the CSF and this implies that there are barrier mechanisms in the plexus between the

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blood and the CSF.” His work on the metabolic regulation of P-32 transport across the plexus lends strong support to this presumption.

As regards metabolic studies of the choroid plexus, early studies by Stiehler and Flexner showed the “indophenol oxidase” activity of the epithelial cells of the plexus to be high. Krebs and Rosenhagen also found that surviving choroid plexus of the cat exhibited a rate of oxygen consumption comparable to that of parenchymatous organs such as liver and kidney. This work was advanced by Friedenwald et al., who found indications of significant activity of certain respiratory enzymes in the choroid plexus. Leduc and Wislocki, using histochemical techniques, found further evidence that the cells of the choroid plexus as well as other parts of what they termed “the hematooencephalic barrier” were quite rich in phosphatases and succinic dehydrogenase. These authors felt that this intense activity in the choroid plexus supported the theory of secretion rather than dialysis.

In order to gain a better understanding of the possible role of the choroid plexus in the formation of cerebrospinal fluid, an extensive study of the cellular chemical and enzymic activity of this tissue was carried out. Enzymes studied that may play roles in active transport included alkaline phosphatase, carbonic anhydrase, cholinesterase, and β-glucuronidase. Those enzyme systems studied which underlie basic cellular metabolism included anaerobic glycolysis, succinic dehydrogenase, and cytochrome oxidase.

MATERIALS AND METHODS

The cats were sacrificed by exsanguination under Nembutal or nitrous oxide anesthesia. The brain was removed rapidly and the choroid plexus was dissected out.

In the histochemical studies, alkaline phosphatase was determined according to the method of Gomori, succinic dehydrogenase by the neotetrazolium method of Rosa and Velardo, and carbonic anhydrase by the method of Kurata.

For the biochemical studies, the choroid plexus was weighed, homogenized in a 2.0 ml. conical McShan-Erway all-glass homogenizer with cold 0.15 M KCl and diluted so that the final homogenate was 5 per cent with respect to tissue. Alkaline phosphatase was measured by a modification of the method of Bessey et al. using 2-amino-2-methyl-1-propanol as the buffer. Five c.mm. of a 0.5 per cent homogenate were incubated with 1 ml. of buffered substrate for 20 minutes at 38°C. Succinic dehydrogenase and cytochrome oxidase were determined by the method of Schneider and Potter using micro-Warburg flasks with an incubation volume of 1.0 ml.

Anaerobic glycolysis was determined according to the method of Reif et al. using the micro-Warburg flasks.

Carbonic anhydrase was measured according to the method of Ashby and Chan. Cholinesterase was determined by the colorimetric method of McOsker and Daniel using acetylthiocholine as the substrate. Beta-glucuronidase was determined according to the method of Fishman et al. Hemoglobin was measured by the method of Crosby and Furth.

The effect of Diamox on the content of carbonic anhydrase of the choroid plexus and the blood was studied. Doses ranged from 1.5 to 25 mg. per kg. of body weight. The cats were anesthetized with intravenous Nembutal (30 mg./kg. body weight)
and a sample of blood was analyzed for hemoglobin and carbonic anhydrase. A catheter was then placed in the cisterna magna and attached to a 1 cc. pipette clamped horizontally at the level of the cistern. Fluid was collected and the flow was measured at 10-minute intervals. A steady state of flow was obtained before the drug was administered intravenously. Studies of the flow were made 1 hour before and after the administration of Diamox. The animal was then sacrificed and the choroid plexus, a sample of blood, and a kidney were studied.

To study the effect of increased intracranial pressure on the content of enzymes in the choroid plexus, kaolin (0.5–1.0 cc.) was injected into the cisterna magna of a cat after the initial pressure had been determined under Nembutal anesthesia. At the end of 1 month the cat was anesthetized as previously and the lateral ventricle of either side was tapped and the pressure was recorded. The brain and a kidney were removed. Enzyme studies were then carried out on the choroid plexus and kidney.

RESULTS AND DISCUSSION

A. Base-line Enzyme Studies on the Cat. Histochemically the choroid plexus of the cat is a simple structure. Schematically it may be considered to consist of a blood vessel surrounded by loose connective tissue which is capped by modified epithelial cells, the ependyma. The blood vessels are mostly capillaries and have the highest concentrations of alkaline phosphatase in their walls (Fig. 1). The red blood cells of course show carbonic anhydrase, while very little enzyme activity or storage of glycogen was
Fig. 2. Succinic dehydrogenase stains only in the ependymal cells. ×55.

Fig. 3. Same as Fig. 2. ×350.
found in the stroma. The ependymal cells lying on the ventricular surface have heavy concentrations of succinic dehydrogenase (Figs. 2 and 3) and carbonic anhydrase (Fig. 4). We did not attempt to separate the layers of the choroid plexus as did Friedenwald et al., but used histochemical techniques to locate enzyme activity which was then quantitated by biochemical methods. Table 1 summarizes our results. The metabolic activity of the whole plexus as measured by anaerobic glycolysis, succinic dehydrogenase, and cytochrome oxidase is about one-third to one-half that of kidney. Considering the fact that the plexus contains a high proportion of connective tissues and blood vessels which contain very little of the above enzymes, it is apparent that the epithelial cells of the plexus may be just as active as those of the kidney. We were impressed with the carbonic anhydrase values of the plexus being almost one-half that of the peripheral blood. The carbonic anhydrase values found in the plexus are higher than those reported by Ashby and Chan in various areas of the cortex of the cat. The high concentration of carbonic anhydrase exclusive of that in the blood has led us to pursue studies on the effect of altering this activity.

B. Diamox Effects on the Cerebrospinal Fluid Flow of the Cat. Tschirgi and his associates reported in 1954 that the rate of formation of cerebro-

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Fig. 4. Carbonic anhydrase stains black in both ependymal cells and blood vessels. X55.
spinal fluid could be decreased radically by a carbonic anhydrase inhibitor, Diamox (2-acetylamino-1,3,4 thiadiazole-5-sulfonamide sodium), when administered intravenously at a level of 150 mg./kg. body weight. This also resulted in a marked reduction in intracranial pressure. Kister\textsuperscript{12} in 1956 confirmed Tschirgi's findings and found that doses as low as 0.5 mg. per kg. could have the same result. However, Knopp \textit{et al.}\textsuperscript{13} have recently reported that there was an immediate increase in cerebrospinal fluid pressures in normal cats and monkeys after Diamox had been given, but that the ultimate pressure was reduced 25–60 per cent below the initial level. They postulated that this rise is caused by increased volume of intracranial vascular structures secondary to an elevated serum CO\textsubscript{2}. This thought has also been stated by Mithoefer.\textsuperscript{19}

With our knowledge of a high concentration of carbonic anhydrase in the choroid plexus over and above that in the blood of the plexus, we proceeded to study the effect of Diamox on the carbonic anhydrase of both the plexus and the peripheral blood, as shown in Table 2. Parallel studies were made on the rate of flow of cerebrospinal fluid. The data indicate that the carbonic anhydrase activity of blood is related inversely to the dosage of Diamox in a straight linear relation. However, carbonic anhydrase activity of the choroid plexus is inhibited to the same degree regardless of the dose of the drug used. The flow is also inhibited to the same extent. This suggests that there may be a barrier to the penetration of Diamox into the cells of

### TABLE 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Choroid Plexus</th>
<th>Kidney</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic glycolysis</td>
<td>4,280 ± 400 (11 animals)</td>
<td>10,000 ± 600 (15)</td>
<td>Microliters CO\textsubscript{2}/hour/gram</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>5,760 ± 380 (13)</td>
<td>18,540 ± 1,600 (8)</td>
<td>Microliters O\textsubscript{2}/hour/gram</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>25,500 ± 1,410 (15)</td>
<td>90,600 ± 30,600 (6)</td>
<td>Microliters O\textsubscript{2}/hour/gram</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>1,767 ± 96 (26)</td>
<td>3,730 ± 804 (7)</td>
<td>Micromoles P-nitrophenyl phosphate split/hour/gram</td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>19.3 ± 3.7 (10)</td>
<td>15.6 ± 2.2 (7)</td>
<td>Micromoles acetylthiocholine split/hour/gram</td>
</tr>
<tr>
<td>β-glucuronidase</td>
<td>950 ± 77 (10)</td>
<td>2,780 ± 500 (6)</td>
<td>Micrograms phenolphthalein glucuronide split/hour/gram</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>179 ± 12 (56)</td>
<td>100.1 ± 12.3 (15)</td>
<td>456 ± 9.0 Ashby units/gram tissue</td>
</tr>
</tbody>
</table>

* Correction for blood.
the plexus, and over the dosage range in these experiments the concentration of Diamox within the cells did not vary.

C. Experimental Hydrocephalus. All animals that survived 1 month showed a two- to eight-fold elevation of ventricular pressure and enlargement of the ventricles with a “wet brain” caused by the injection of kaolin in the cisterna magna producing obstructive hydrocephalus. The concentrations of carbonic anhydrase, succinic dehydrogenase, and alkaline phosphatase were not altered in a total of 16 animals studied.

D. Clinical Application. Basic knowledge of the biochemical activity of the choroid plexus is necessary if advances are to be made in the management of intracranial fluid problems such as hydrocephalus. Isotope studies lead one to assume that the choroid plexus is certainly not the only factor in elaboration of the fluid; yet, its histological structure seems so well designed

| TABLE 2 |
| Effect of Diamox |

<table>
<thead>
<tr>
<th>Studies</th>
<th>Dosage in mg./kg. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td></td>
</tr>
<tr>
<td>Per cent blood inhibition</td>
<td>91</td>
</tr>
<tr>
<td>Per cent choroid plexus inhibition</td>
<td>73</td>
</tr>
<tr>
<td>Rate of flow</td>
<td></td>
</tr>
<tr>
<td>30 min. before Diamox</td>
<td>0.70 cc.</td>
</tr>
<tr>
<td>30–60 min. after Diamox</td>
<td>0.28 cc.</td>
</tr>
<tr>
<td>Per cent inhibition flow</td>
<td>60.00</td>
</tr>
</tbody>
</table>

dosage for active or passive transfer of constituents of blood that one can hardly discount the possibility that it produces or absorbs cerebrospinal fluid. The work presented indicates the extensive role of the ependymal cells of the plexus; one can see a parallel in studies of the kidney.

This work has been applied to man in a limited fashion. An indwelling polyethylene catheter was placed in the ventricle of a 3-month-old infant with hydrocephalus (Fig. 5). The weight was 13 kg. A meningomyelocele had been repaired at birth with subsequent enlargement of the head. Baseline observations were made on the pressure. Five hundred mg. of Diamox were then administered intravenously with marked reduction of the pressure. The study was repeated with a decrease in pressure, not so marked. A daily dose of 250 mg. of Diamox orally was then given and the fontanelle remained soft until the child died of intercurrent infection 3 months later.

In another case, a glioblastoma multiforme had been removed from the left frontal lobe of a 53-year-old man. The tumor was found to pass across the corpus callosum to the right side. A rapid recurrence of increased intracranial pressure appeared and he became comatose. The bone flap was tense
and the ventricular pressure was 280 mm. of cerebrospinal fluid. He was
given 2 gm. of Diamox intravenously and within 4 hours the ventricular
pressure was 460 mm. of cerebrospinal fluid. However, he was found to be
alert within 8 hours and his ventricular pressure was 140 mm. He was main-
tained on 3 gm. of the drug daily after this and his pressure was under 130
mm. for 2 weeks. He was discharged to a nursing home without any drug
therapy and died within 1 month.

This case certainly bears out the warning of Knopp et al.,\textsuperscript{13} that there may
be a rise in the intracranial pressure after the drug is started; but our work
has been concerned primarily with the reduction in pressure after the initial
rise.

The cases presented seem to indicate that increased intracranial pressure
may be reduced by the use of Diamox. Because of the effect of Diamox as a
diuretic, we are aware that its effect on cerebrospinal fluid pressure may in-
volve certain extracranial structures. From our work presented, one cannot
yet assume that carbonic anhydrase activity of the choroid plexus plays any
major role in the production of cerebrospinal fluid. The enzyme activity
found in the ependymal cells indicates that a very high degree of work is
being performed for some as yet unknown reason.

**SUMMARY**

1. Preliminary observations on the enzyme activity of the choroid plexus
of the cat indicate the following:

a. Histochemically, the ependymal cells of the plexus were found rich in
succinic dehydrogenase and carbonic anhydrase, while the blood vessels had
high concentrations of alkaline phosphatase.

b. Biochemical analysis of the choroid plexus indicates that general
metabolic activity is one-third to one-half the value of the kidney.

2. Diamox, a carbonic anhydrase inhibitor, reduces the flow of cerebro-
spinal fluid regardless of the dose of the drug used in this study. Direct cor-

![Fig. 5. Study of intraventricular pressure in a hydrocephalic infant treated with Diamox.](image-url)
relation existed between the dosage of the drug and carbonic anhydrase activity of the blood; but flow of cerebrospinal fluid was not directly correlated with inhibition of carbonic anhydrase of the choroid plexus, although inhibition occurred with very small dosages of the drug used.

The authors wish to acknowledge preliminary histochemical studies made by three Dartmouth medical students—Mr. Bruce Gilmore, Mr. Ross McIntyre, and Mr. Lawrence Seymour. These men were supported by Lederle Company research fellowships.

REFERENCES
19. Metahefer, J. R. Personal communication.

DISCUSSION

Dr. A. Earl Walker: I don't intend to discuss the scientific content of these papers because the biochemical aspects are far beyond my competence. I do, however, wish to point out the complexity of the problems that are involved in this approach to hydrocephalus. Dr. Fisher and his associates are certainly to be congratulated upon their attempt to get at the basic factors involved in the production of spinal fluid. When one realizes that changes in many factors operating inside the cranial cavity, such as arterial blood pressure, cerebral blood flow, the osmotic pressure of the blood, as well as the actual blood volume within the intracranial cavity, may modify greatly the production of spinal fluid, one can understand the complexity of the problem. When this is compounded by the indirect factors such as those modifying kidney function, by which more fluid, water in particular, is excreted, washing out electrolytes which may modify adrenal function and respiratory mechanisms, we see that the whole problem is still much more complicated.

Dr. Fisher, in his conclusions, wisely recognizes that at this time one cannot state that carbonic anhydrase is the answer to the production of the spinal fluid by the choroid plexus. We know that spinal fluid may be produced, particularly the water component, by many other portions of the central nervous system, the arachnoid and the ependyma, so that other enzyme systems may be implicated.

Dr. Bertram Selverstone: I am most grateful to Drs. Fisher and Copenhaver for helping to rehabilitate the choroid plexus from the rather sad state in which it has been left by some of those who studied the cerebrospinal fluid with isotopes.

I should like to show three slides which indicate that our isotope studies, at least, have emphasized the importance of the choroid plexus in the formation of the cerebrospinal fluid. Our results are quite in keeping with those reported today.

[Slide] These data were obtained in a patient whose ventriculogram you see here, a young man of 20 with arrested hydrocephalus, a normal cerebrospinal fluid pressure, and the very large ventricles shown in the slide.

[Slide] Two ventricular needles were placed, as you can see, one in the anterior horn, far in front of the foramen of Monro and the choroid plexus, and another close to the glomus of the choroid plexus. The patient was then given a dose of K\(^{42}\) intravenously and very small samples were taken from each ventricular needle.

[Slide] These were the curves obtained. The light lines show the concentration of the radioactive water injected at the same time as the K\(^{42}\). These curves are practically identical whether near the choroid plexus or far from it. In the anterior horn, i.e., away from the choroid plexus, one heavy line shows that there is a very slow rise in the K\(^{42}\) concentration, so that the curve at first is concave upward. Near the choroid plexus in the atrium, the K\(^{42}\) concentration rises very rapidly indeed, showing that the exchange of K\(^{42}\) is very much faster in the immediate vicinity of the plexus than in the anterior horn, to which some K\(^{42}\) must apparently diffuse or flow before its concentration begins to rise rapidly. The two curves meet at about 90 minutes.

We have similar data for sodium 24 which show the same thing. I think that the evidence from tracer experiments is quite in keeping with that of Drs. Fisher and Copenhaver; the choroid plexus must have importance in the exchange of the ionic constituents of the cerebrospinal fluid.