tion of the tissue that is washed out of $^{85}$Kr during the first few seconds of the clearance curve. The results of the diffusion model indicate that this tissue is limited to a narrow strip consisting of two thirds of the choroid plus approximately the outer one fifth of the retina. This tissue mass will be constant over a wide range of flows. The numerical value of this mass is important only when this method of obtaining absolute values of blood flow is compared with other methods of blood flow measurement.

The authors wish to thank Dr. A. M. Harper, Reader in Surgical Physiology, Wellcome Surgical Research Institute, for his encouragement and advice and the technical staff of the laboratory for their invaluable assistance. We also wish to thank Professor W. S. Foulds for his encouragement, direction, and the interest he has shown in the project.

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Key words: cerebral blood flow, choroidal blood flow, baboon, krypton, xenon, diffusion.

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The response of the choroidal and cerebral circulations to changing arterial $P_{CO_2}$ and acetazolamide in the baboon.


Having established control values for choroidal and cerebral blood flow in twelve baboons, the response of both circulations to changing arterial $P_{CO_2}$ and intravenous acetazolamide was studied. The blood flow in both circulations varied directly with the $P_{ACO_2}$, the magnitude of the response being very similar. There was a 3.6 percent change in both choroidal and cerebral blood flow per millimeter of mercury change in $P_{ACO_2}$. Intravenous acetazolamide (25 mg./kg.) produced an increase in flow lasting approximately 50 minutes in both cerebral and choroidal circulations.

Since the initial observations by Reivich, it has now been well established that alterations in $P_{CO_2}$ have profound effects on cerebral blood flow, and it is now thought that CO$_2$ reactivity is mediated by pH changes in cerebrospinal fluid around the arteries. A number of authors have also described changes occurring in choroidal blood flow and volume with $P_{ACO_2}$ in animals. In 1956 Betterman and Fellows found a marked and consistent increase in choroidal blood volume in cats on administration of 8 to 10 percent carbon dioxide. Friedman and Chandra (1972) noted an increase in choroidal blood flow with administration of carbon dioxide. Strang, Wilson, and Johnson showed an increase in rabbit choroidal blood flow with increasing $P_{ACO_2}$ and correlated changes in flow with measurements of the arterial $P_{CO_2}$.

After an initial observation by Mitthoefer, Mayer, and Stocks, a number of authors have described the cerebral vasodilator effects of intravenous acetazolamide in both animals and man. In addition to its action as an ocular hypotensive agent, Macri and Brown have described a vasoconstrictor action of acetazolamide on the anterior uvea. As far as we are aware, little is known of its possible effects on choroidal blood flow. In this experimental study, the response of cerebral and choroidal blood flow, under identical conditions of blood gas tensions and systemic blood flows...
Pressure, to changing \( P_{\text{ACO}_2} \), and intravenous acetazolamide is described, and possible mechanisms of action are discussed.

**Materials and methods.** The experiments were performed on 12 baboons (Papio anubis) weighing between 5 and 10 kg. The experimental methods were those outlined in Strang, Wilson, and MacKenzie, with the following differences:

1. In six animals, step hypercapnia was achieved by introducing \( CO_2 \) into the respired gas mixture, and hypocapnia by hyperventilation.

2. Measurements of cerebral and choroidal blood flow were made at various time intervals up to 2 hours after intravenous acetazolamide (25 mg./kg.) in a further six animals. The increase in \( P_{\text{ACO}_2} \), which occurred following intravenous acetazolamide injection was compensated for by adjusting the respirator to maintain normocapnia. \( P_{\text{ACO}_2} \) was measured on femoral artery blood samples using a direct-reading electrode system (Corning, Eel).

3. The intraocular pressure was monitored continuously via a 30-gauge needle in the anterior chamber during those experiments which assessed the response to changing \( P_{\text{ACO}_2} \). During the experiments in which the response to acetazolamide was being measured, a second 30-gauge needle was inserted into the anterior chamber. This was connected to a saline reservoir, and the intraocular pressure maintained between 12 and 15 mm. Hg.

**Results**

**Response to changing arterial \( P_{\text{CO}_2} \).** The response of the cerebral blood flow to changing \( P_{\text{ACO}_2} \) is shown in Fig. 1. The results are expressed as a percentage increase or decrease in flow from normocapnia.
Fig. 3. Responses of both cerebral and choroidal circulations over 50 minutes following injection of intravenous acetazolamide (25 mg./kg.).

The increase in cerebral blood flow reached a peak 15 to 20 minutes following the injection of acetazolamide. The response of the choroidal blood flow was more variable in that peak flow values occurred at different times after the acetazolamide injection in each animal, but in all the experiments an increase in flow occurred during the 50 minutes after injection. Flow values returned to control levels after 50 minutes. If one groups both sets of values for comparison (Fig. 3), there is a large scatter since the response is time related but there is a mean increase in choroidal blood flow of 38 percent and a mean increase in choroidal blood flow of 75 per cent, both values being significantly different from the base line values (p <0.05).

Discussion. These experiments confirm the established phenomenon that cerebral blood flow varies directly with the \( P_{ACO_2} \). The correlation coefficient of the regression is 0.81 (p <0.001).

Fig. 2 shows the response of the choroidal circulation to changing \( P_{ACO_2} \). Twenty-five measurements of flow were made. The response of the choroidal circulation was very similar to that of the cerebral circulation. The correlation coefficient of the regression was 0.69 (p <0.001).

If one uses these results to calculate the percentage change in flow per millimeter of mercury change in arterial Pco2, one obtains a figure of 3.6 percent for both choroidal and cerebral circulations.

Response to intravenous acetazolamide. The increase in cerebral blood flow reached a peak 15 to 20 minutes following the injection of acetazolamide.

It is now widely thought that the CO2 cerebrovascular sensitivity is mediated by pH variations in the cerebral interstitial fluid. Changing intravascular pH in the presence of an intact blood-brain barrier does not alter cerebral blood flow, since the blood-brain barrier is relatively impermeable to hydrogen ion. Since a blood-retinal barrier also exists, it is reasonable to assume that a similar mechanism may account for the retinal vascular response. Studies on both the cerebral and retinal vasculature have shown that this...
Fig. 4. Possible mechanisms of action of changing intravascular Pco₂ on brain and retinal flow in the presence of a blood-brain and blood-retinal barrier. Acetazolamide blocks the reaction CO₂ + H₂O ⇌ H₂CO₃ as shown. R-H⁺, Any acid metabolite; L.-H⁺, lactic acid; E.C.F., extracellular fluid.

In the choroid, however, no such barrier exists, and small molecules such as sodium fluorescein diffuse rapidly into the extravascular compartment. There is, nevertheless, a barrier between the choroid and the tissue it supplies, namely, the outer retina, at the level of the retinal pigment epithelium. It may be that the reduction in pH which accompanies increasing Pₐₙₑ₉ plays a role in the increase in choroidal blood flow, and this may be combined with a mechanism similar to that mediating the cerebral blood flow response since pH will change in outer retina with changing Pₐₙₑ₉. The principal difference between brain and inner retina and the choroid and outer retina is that a chorioretinal barrier exists, not at the level of the capillary endothelium but at the retinal pigment epithelium, although this does not rule out the importance of changes in intravascular pH as being an important factor in the regulation of choroidal blood flow. Fig. 4 shows a diagram of the possible mechanism of action in brain and retina in their responses to changing Pₐₙₑ₉. It is as yet not clear how pH variations in the cerebral interstitial fluid influence the tone of smooth muscle cells, but it is probable that pH variations inside these cells are the important factor.

Intravenous acetazolamide results in an increase in Pₐₙₑ₉ and a reduction in pH during fixed ventilation. In these experiments the change in Pco₂ was minimized by hyperventilation, and the Pₐₙₑ₉ maintained at normocapnic levels.

Acetazolamide can lower the intracocular pressure but a reservoir maintained the intraocular pressure between 12 and 15 mm. Hg in these experiments. An increase occurred in both cerebral and choroidal blood flow, reaching a maximum after 15 minutes with the cerebral blood flow; the peak response was more variable from experiment to experiment with the choroidal blood flow. Although, as mentioned previously, the reduction in arterial pH which occurs with acetazolamide may have a direct effect on the choroidal circulation as in the response to CO₂, another possible mechanism is shown in Fig. 4. Acetazolamide blocks the reaction, as follows:

\[
\text{Acetazolamide} + \text{CO}_2 \rightarrow \text{H}_2\text{CO}_3
\]

This means that this route is no longer available for the disposal of metabolically produced H⁺, which is then free to act on the smooth muscle cells of the arterioles, causing vasodilatation and increased flow. It is of interest that doses of acetazolamide of 10 to 15 mg./kg. have no effect on cerebral blood flow in our experience. A dose of 25 mg./kg. is necessary to produce an increase in flow. This may mean that if the blood-brain
barrier is relatively impermeable to acetazolamide, a high intravascular concentration must be achieved before significant levels occur in the extracellular fluid. This hypothesis depends on the assumption that carbonic anhydrase is present in the tissue fluid, and although carbonic anhydrase inhibition is the sole action of acetazolamide, the mechanism whereby it causes an increase in flow may be much more complex. Since brain, choroid, and retina respond in such a similar way to changing PACC, one might expect the retinal circulation to show vasodilatation and increased flow after intravenous acetazolamide, and this is being investigated currently.

As the behavior of all these vascular beds is so similar in their response to changing PACC, one might expect them to behave in a similar way in other pathophysiological states. This is not surprising when one considers that the eye develops as an extension of the brain. The authors wish to thank Dr. A. M. Harper, Reader in Surgical Physiology, Wellcome Surgical Research Institute, for his encouragement and advice and the technical staff of the laboratory for their invaluable assistance. We also wish to thank Professor W. S. Foulds for his encouragement and direction, and the interest he has shown in the project. The authors wish to express their appreciation for the constructive criticism of Dr. Kyuye Kogure, Department of Neurology, University of Miami, Miami, Fla., and his help in constructing Fig. 4.

From the *Tennent Institute of Ophthalmology, University of Glasgow; **Department of Clinical Physics and Bio-Engineering, West of Scotland Health Boards, Glasgow; and ***Wellcome Surgical Research Institute, University of Glasgow. This work was supported by Medical Research Council grant G.971/366/C. E. MacKenzie is supported by Tenovus (Scotland). Submitted for publication Sept. 17, 1976. Reprint requests: Dr. T. M. Wilson, Department of Ophthalmology, University of Western Australia, Box 229, West Perth 6005, Western Australia.

Key words: choroidal blood flow, cerebro blood flow, baboon, arterial Pco2, acetazolamide.

Ocular measurement by simple gravimetric methods. Roger C. Wales.*

A rapid, precise, and easy method for measuring ocular volume would be useful in estimating the theoretical tension changes involved in the relation of pressure and volume changes and in estimating areas of different membranes of the eye. Rabbit eyes were weighed in air and then in water, and the mass and volume of each eye was calculated. For eyes between 2.4 and 3.4 gm., the volume of the eye was given by (mass × 0.97 - 0.02) ml. The difference between paired eyes was always less than 1% (i.e., less than 100 nig.). If a spherical eye is assumed, the 'mean radius' could be calculated to 0.1 mm. from the volume (for tension calculations). From the depth of various segments of the eye, areas could be calculated. If a destructive experiment were to be performed on one live eye, the other could be used as a control for measurement after the conclusion of the experiment.

In deriving an expression for the relation of changes in pressure to changes in volume of the eye (i.e., rigidity), based on the mechanical properties of the eye coats, Kearns1 showed that even if the modulus of elasticity of the sclera in two eyes was the same, the relation was dependent upon the mean radius of the eye and the thickness of the sclera. This was assuming a spherical eye, although in a real eye a defini-

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