

# The Effects of Acetazolamide on Cerebral Blood Flow and Cerebral Tissue $P_{O_2}$

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An experiment was designed to rule out hypoxia of cerebral tissue as a mediator of increased cerebral blood flow (CBF) due to acetazolamide (Diamox®). Hypoxia might be produced by carbonic anhydrase inhibition by delaying the acidification of the red blood cell until it left the tissue capillary.

Fifteen dogs were studied under pentobarbital anesthesia. CBF was estimated from the arteriovenous oxygen content difference across the brain. Cerebral tissue oxygen tension ( $P_{T_{O_2}}$ ) was measured directly on the cortical surface. CBF was altered by altering arterial  $P_{CO_2}$  ( $P_{a_{CO_2}}$ ) before and after the intravenous administration of 25 mg./kg. acetazolamide. At  $P_{a_{CO_2}} = 30$  mm. Hg, acetazolamide increased CBF by 69 per cent; at  $P_{a_{CO_2}} = 40$  mm. Hg, by 64 per cent; at  $P_{a_{CO_2}} = 50$  mm. Hg, by 55 per cent. This was accompanied by an increase in  $P_{T_{O_2}}$  of 20 mm. Hg at  $P_{a_{CO_2}} = 30$  mm. Hg; 19 mm. Hg at  $P_{a_{CO_2}} = 40$  mm. Hg; 16 mm. Hg at  $P_{a_{CO_2}} = 50$  mm. Hg. Since  $P_{T_{O_2}}$  increased, clinical use of the drug in association with mechanical ventilation in the treatment of chronic hypercapnia appears justified. The effect of acetazolamide on cerebrovascular tone remains unexplained.

ACETAZOLAMIDE (Diamox®), a carbonic anhydrase inhibitor, has been shown to increase cerebral blood flow (CBF). Mithoefer and his colleagues first demonstrated this effect in dogs.<sup>1</sup> Similar observations subsequently have been recorded both in monkeys<sup>2</sup> and in

man.<sup>3,4</sup> The mechanism by which acetazolamide increases cerebral blood flow has not been clarified adequately, however. Currently, we use acetazolamide in our intensive care unit<sup>5</sup> to offset the fall in CBF that occurs when patients with chronic hypercapnia are artificially ventilated to a lower  $P_{CO_2}$ . The theoretical possibility arose that cerebral tissue hypoxia may be associated with the use of acetazolamide through an inhibitory action on the Bohr effect in the tissue capillaries. The clinical use of acetazolamide would not be justified if tissue hypoxia accompanies the cerebral vasodilation.

The following experiments were designed to rule out the presence of cerebral hypoxia after acetazolamide administration. Furthermore, we hoped that more information about the mechanism by which acetazolamide increases CBF could be gained.

## Methods

Fifteen mongrel dogs were studied under pentobarbital anesthesia (30 mg./kg. intravenously). The trachea was cannulated, and positive-pressure respiration with room air was instituted with a Bird Respirator, Mark 7. Temperature was monitored with an esophageal thermistor. The femoral vein and artery were cannulated on one side. The dog then was placed in the prone position, and the head was fixed to an operating table frame. A 19-gauge needle was introduced into the sagittal sinus through a tight-fitting midline drill hole heading 45° caudally in the sagittal plane. The cerebral cortical surface was exposed by opening a burr hole, 13–15 mm. in diameter, in the frontoparietal area, about 1–2 cm. on either side of the midline, and removing the underlying dura. A Radiometer blood  $P_{O_2}$  electrode, removed from its cuvette, was used to record cortical surface  $P_{O_2}$  ( $P_{T_{O_2}}$ ) continuously. A 6  $\mu$  mylar membrane was used, with

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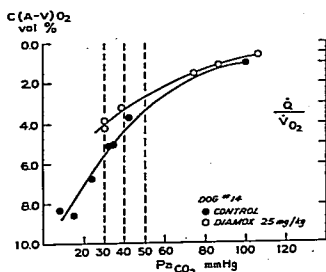


FIG. 1. Effect of Diamox on cerebral blood flow. Graphic handling of individual data for a single dog. Best fit lines were drawn by eye representing the relationship of the A-V<sub>O<sub>2</sub></sub> difference to arterial P<sub>CO<sub>2</sub></sub>. The values of A-V<sub>O<sub>2</sub></sub> read from these lines at P<sub>CO<sub>2</sub></sub> = 30, 40 and 50 were averaged for all dogs. The reciprocal of the A-V<sub>O<sub>2</sub></sub> difference, plotted as the right ordinate, has the units of blood flow per ml of oxygen consumption by the tissue through which the blood flowed. This is herein termed the "flow equivalent" by the analogy with "ventilation equivalent," which is the expired ventilation divided by the oxygen consumed from that volume.

a 3 mm.-diameter disc of 12  $\mu$  cellophane acting as spacer between the mylar and the platinum/glass tip. This combination of membranes has four noteworthy effects on the electrode's performance. 1) The area of electrode surface sensitive to oxygen is widened, in effect integrating tissue surface P<sub>O<sub>2</sub></sub> over an area of several square mm., reducing variability that is due to its relation to the surface vessels. 2) The so-called stirring effect is virtually eliminated; the electrode's output in unstirred liquid equals the output in the gas with which the liquid is equilibrated. When used on tissue surfaces, the diffusion gradient is confined to the membrane almost totally. The electrode may then be assumed not to reduce the local tissue P<sub>O<sub>2</sub></sub> by consuming its oxygen. 3) Response time to 99 per cent is delayed to about two minutes. 4) Output current is at least ten times greater when mylar and cellophane are used than that obtained with mylar without underlying cellophane, and still several times less than that obtained with polypropylene 25  $\mu$  thick. This electrode was firmly clamped in the burr hole, its tip touching the cortex and usually depressing it about 1 mm. at the center. The

electrode and cortical temperatures were held at 37° C. by blowing air thermostated to 37° C. over the field from a 1,500-watt heater. A thermistor probe mounted adjacent to the electrode, working through a temperature controller relay (Yellow Springs), cycled the heater element while the fan was in action. Drying of the cortical surface was avoided by making the burr hole only large enough to accept the electrode tip. Once *in situ*, the electrode was not removed until the end of the experiment. Calibration was achieved in distilled water at 37° C., through which nitrogen and room air were bubbled. This was done once just prior to placing the electrode on the cortical surface, and at the end of the experiment. P<sub>TO<sub>2</sub></sub> was corrected for electrode drift by assuming constant drift rate between calibrations. The correction during the duration of an experiment did not exceed 10 per cent.

Arterial blood pressure (Statham transducer no. P23AC) and P<sub>TO<sub>2</sub></sub> were continuously recorded (the latter with a servo-pen having a 30 cm. paper scale) on a Gilson polygraph. Paired arterial and sagittal venous blood samples were drawn into heparinized syringes. Each sample consisted of 2-4 ml. of blood. Venous samples were drawn slowly over 30-60 seconds per sample. Arterial and venous bloods were analyzed immediately for P<sub>O<sub>2</sub></sub>, P<sub>CO<sub>2</sub></sub>, pH and oxygen saturation. Corrections were made for temperature<sup>6</sup> and for the "stirring" effect (+5 per cent), that is, the error in the blood P<sub>O<sub>2</sub></sub> electrode due to lack of stirring.<sup>7</sup>

Blood hemoglobin concentration was measured in duplicate in each dog. Arterial P<sub>CO<sub>2</sub></sub> was adjusted to low, normal, and high levels, both before and after the intravenous administration of 25 mg. acetazolamide per kg. body weight. This was achieved either by hyperventilation or by adding CO<sub>2</sub> to the inspired mixture. Paired arterial and venous samples were drawn at each setting at least 20 minutes after altering arterial P<sub>CO<sub>2</sub></sub>, and always at least 10 minutes after P<sub>TO<sub>2</sub></sub> reached a steady plateau. No measurements were attempted unless the systolic blood pressure was at least 120 mm. Hg.

The dog's temperature was maintained between 35° and 39° C.

Calculations

Cerebral oxygen consumption,  $\dot{V}_{O_2}$ , according to the principle of conservation of matter (the Fick equation) must equal cerebral blood flow,  $\dot{Q}$ , times the arteriovenous oxygen content difference,  $C(A-V)_{O_2}$ . The equation may be written thus:

$$1/C(A-V)_{O_2} = \dot{Q}/\dot{V}_{O_2}$$

Therefore, the reciprocal of  $C(A-V)_{O_2}$  is the blood flow in ml. per ml. of oxygen consumption from that blood. We term this ratio the flow equivalent, by analogy with ventilation equivalent ( $\dot{V}_E/\dot{V}_{O_2}$ ). The normal value for cerebral flow equivalent in unanesthetized man is 14 (CBF 42 ml./100 gm./min.,  $\dot{V}_{O_2}$  3 ml./100 gm./min.).

Arterial  $P_{CO_2}$  ( $P_{aCO_2}$ ) and the arteriovenous oxygen content difference [ $C(A-V)_{O_2}$ ] were plotted for each experiment before and after acetazolamide administration (fig. 1). Two curves were drawn by estimation relating the observed points before and after acetazolamide. The right ordinate of figure 1 is the reciprocal of  $C(A-V)_{O_2} \times 100$  which is the flow equivalent.

From the curves thus plotted, values for  $\dot{Q}/\dot{V}_{O_2}$  were read for each dog at  $P_{aCO_2}$  levels of 30, 40, and 50 mm. Hg before and after

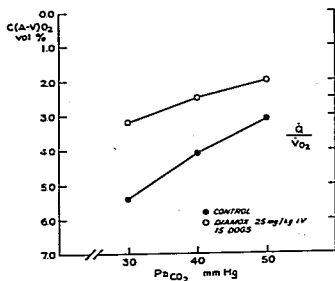


FIG. 2. Effect of Diamox on cerebral blood flow. Mean A-V<sub>O<sub>2</sub></sub> differences before and after acetazolamide at three levels of  $P_{aCO_2}$  in 15 dogs. The right ordinate is flow equivalent, which at constant O<sub>2</sub> consumption is proportional to flow. The normal value in man is 14 (flow = 42 ml./100 gm./min.; O<sub>2</sub> consumption = 3 ml./100 gm./min.).

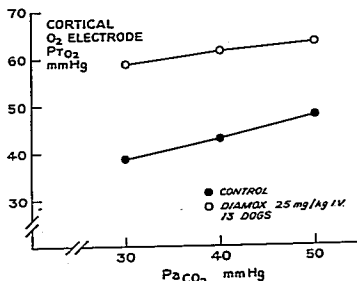


FIG. 3. Effect of Diamox on cerebral O<sub>2</sub> tension. Mean cortical surface  $P_{rO_2}$  in 13 dogs before and after acetazolamide as a function of arterial  $P_{CO_2}$ .

acetazolamide administration. Similarly, the relationship between  $P_{rO_2}$  and  $P_{aCO_2}$  was evaluated before and after acetazolamide.

Results

Fourteen dogs showed increased CBF\* after acetazolamide at each level of  $P_{aCO_2}$  tested. One dog showed no change in flow. The mean increase in flow was 69 per cent at  $P_{aCO_2}$  30 mm. Hg, 64 per cent at  $P_{aCO_2}$  40 mm. Hg, and 55 per cent at  $P_{aCO_2}$  50 mm. Hg. The increase in flow was statistically significant using paired analysis and Student's *t* test at all  $P_{aCO_2}$  levels tested ( $P < 0.01$ ) (fig. 2).

Satisfactory cortical  $P_{O_2}$  recordings were obtained in 13 experiments, the electrode having failed or drifted too much in two. Cortical oxygen tension was increased after acetazolamide administration in all dogs at all levels of  $P_{aCO_2}$ . The mean increase in  $P_{rO_2}$  with acetazolamide was as follows: 20 mm. Hg at  $P_{aCO_2}$  30 mm. Hg, 19 mm. Hg at  $P_{aCO_2}$  40 mm. Hg, and 16 mm. Hg at  $P_{aCO_2}$  50 mm. Hg. The increase in  $P_{rO_2}$  was statistically significant ( $P < 0.01$ ) at all  $P_{aCO_2}$  levels tested (fig. 3).

Arterial  $P_{O_2}$  ranged from 70 to 120 mm. Hg.

\* In discussing our results, the term CBF denotes cerebral blood flow equivalent as described in the previous section.

TABLE 1. Effect of Acetazolamide on Cerebral Blood Flow\*

	Control	Acetazolamide
Ventilation (L./min.)	4.6	8.5
Alveolar $P_{CO_2}$ (mm. Hg)	35	14
Arterial $P_{CO_2}$ (mm. Hg)	35	35
Arterial pH	7.41	7.35
Arterial $HCO_3^-$ (mEq/L.)	21.9	19.3
Cortical surface $P_{CO_2}$ (mm. Hg)	45	48
Cortical surface pH	7.22	7.12
Cortical surface $HCO_3^-$ (mEq/L.)	17.3	14.4
CBF with $^{86}Kr$ (ml./gm./min.)	0.87	1.43
CBF with A- $VO_2$ (ml./ml. $VO_2$ )	11.9	28.6

\* Four dogs, trichloroethylene, gallamine and artificial ventilation. Data of Severinghaus, Harper, Ledingham and McDowell.

### Discussion

Although the cerebral vasodilator effect of acetazolamide is now well established, none of the mechanisms suggested so far adequately accounts for the full extent of rise in CBF associated with the drug. Increased  $P_{aCO_2}$  has long been established as a cerebral vasodilator. A thorough review of the subject, together with quantitative data on this relationship in the monkey, has been provided recently by Reivich.<sup>8</sup> Severinghaus and Lassen<sup>9</sup> recorded the time course of changes in CBF after stepwise changes in  $P_{aCO_2}$  in man. They were able to conclude from their data that CBF responds to changes in  $P_{aCO_2}$  (or more likely,  $P_{CO_2}$  of the arteriolar wall), rather than the cerebral tissue  $P_{CO_2}$ . We anticipated from this observation that cerebral blood flow would not increase with acetazolamide if  $P_{aCO_2}$  were held constant, even though tissue  $P_{CO_2}$  rose. Published evidence, however, does not support the conclusion. Gotoh *et al.* found that, although acetazolamide increased  $P_{aCO_2}$  in man, measured with a cuvette on-line, the rise was only 2-3 mm. Hg after intravenous administration of 500 mg. of the drug, whereas the associated rise in CBF was estimated at 30 per cent.<sup>10</sup> Ehrenreich *et al.* reported only a 1-2 mm. Hg rise in  $P_{aCO_2}$  (measured with a  $CO_2$  electrode *in vitro*), whereas CBF increased by 65-76 per cent after the administration of 1.0 gm. of acetazolamide in man.<sup>4</sup> If Reivich's quantitative data<sup>8</sup> in the monkey are applied to the data in man, the increase

in CBF far exceeds that expected from the rise in  $P_{aCO_2}$ .

Similarly, increased tissue  $P_{CO_2}$  ( $P_{TCO_2}$ ) alone cannot be implicated in causing the rise in CBF.

Severinghaus, Harper, Ledingham and McDowell measured CBF after the administration of acetazolamide (25 mg./kg. intravenously) in four dogs. The dogs were artificially ventilated under trichloroethylene and gallamine anesthesia. Cortical surface flow was measured by the Krypton washout technique, and CBF was estimated from arterial-to-sagittal sinus  $O_2$  differences. Table 1 summarizes their findings. A marked rise in CBF was observed even when  $P_{TCO_2}$  was maintained relatively constant by hyperventilation. It seems, therefore, that whether or not a rise in arterial or tissue  $P_{CO_2}$  is involved in the cerebral vasodilation that is due to acetazolamide, this cannot explain the rise in CBF during hyperventilation.

Metabolic acidosis in arterial blood invariably occurs with the administration of acetazolamide owing, at least in part, to bicarbonate diuresis. This effect is noted as early as 10 minutes after acetazolamide administration.<sup>10</sup> It has been shown, however, that arterial acidosis has no cerebral vasodilator effects at constant  $P_{CO_2}$ .<sup>11</sup> The blood-brain barrier seems to delay effectively or prevent acidosis from extending to the cerebral arteriolar smooth muscle. These muscle cells do seem to respond, however, to elevated  $H^+$  levels when the rise is due to  $CO_2$ . Betz and Kozak demonstrated that in the normal state (*i.e.*, uninhibited carbonic anhydrase) cerebral vasodilation due to  $CO_2$  inhalation was correlated best with decreased pH in the cerebral extracellular fluid (ECF).<sup>12</sup> The data of table 1 indicate that significant acidosis of the cortical surface occurred even in the face of an almost constant cortical  $P_{CO_2}$ , after acetazolamide administration in dogs. This metabolic acidosis of the cerebral ECF was associated with an elevated CBF.

The cause of cerebral metabolic acidosis is not apparent; a possible factor might be tissue hypoxia. Cerebral tissue hypoxia could well be expected to raise CBF. Certainly, lowered arterial  $P_{O_2}$  ( $P_{aO_2}$ ) is known to result in a fall

in cerebral vascular resistance.<sup>13</sup> If acetazolamide were to cause cerebral hypoxia, an undesirable effect of the drug would be found that is antagonistic to the primary reason for using acetazolamide during hyperventilation of patients with chronic hypercapnia.

Based on theoretical considerations, cerebral tissue hypoxia should accompany blockade of carbonic anhydrase. Normally, the  $CO_2$  produced by the tissues diffuses into capillary blood and helps to unload oxygen from hemoglobin by acidifying the interior of the red blood cells and by shifting the oxygen dissociation curve to the right (*i.e.*, to a higher  $P_{O_2}$ )—the Bohr effect.<sup>14</sup> Acetazolamide should block this helpful effect of  $CO_2$  on oxygen unloading, because it delays the conversion of  $CO_2$  to  $H^+$  ions by blocking carbonic anhydrase. Total inhibition of the enzyme theoretically would slow the  $CO_2$  hydration reaction 13,000-fold.<sup>15</sup> Acidification of the red blood cell occurs only after blood has left the tissue capillary.

The resultant fall of end-capillary  $P_{O_2}$  may be predicted as follows (fig. 4): Assume an end-capillary cerebral  $O_2$  saturation of 65 per cent,  $P(A-V)_{CO_2}$  across the brain of 10 mm. Hg, and 97 per cent arterial oxygen saturation, arterial pH 7.40, arterial  $P_{CO_2}$  40 mm. Hg, and 15 gm. per cent hemoglobin concentration. Each gram of hemoglobin yields 0.3 mEq. of buffer base<sup>16</sup> when reduced from oxyhemoglobin. Thus, end-capillary blood at 65 per cent saturation would have gained 1.68 mEq. base across the brain. Using Siggaard-Andersen's nomogram,<sup>16</sup> venous pH is calculated to be 7.36. From the  $O_2$  dissociation curve,<sup>6</sup> venous  $P_{O_2}$  is read at 35.7 mm. Hg. The latter value, then, is the expected cerebral end-capillary  $P_{O_2}$  in normal subjects.

After total inhibition of carbonic anhydrase, the pH change that would occur when oxyhemoglobin is reduced depends on its own buffer properties, unaffected by  $CO_2/HCO_3^-$ . This buffer property was evaluated by Rossi and Roughton.<sup>17</sup> They showed that, when 8.8 mEq. of  $CO_2$ -free oxyhemoglobin solution is fully reduced, the pH rises by 0.2 pH units. If only one third of the oxyhemoglobin is reduced (*i.e.*, 97–65 per cent saturation), a 0.06 pH-unit rise would be expected. From this

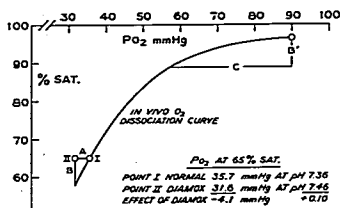


FIG. 4. Predicted effect of Diamox on end-capillary  $P_{O_2}$  ( $O_2$  saturation 65 per cent). Line A represents the predicted fall of  $P_{O_2}$  in cerebral end-capillary blood after acetazolamide. Line B and Line B' represent the predicted fall of  $O_2$  saturation that would be associated in normal blood with a decrease of  $P_{O_2}$  as in line A. Line C represents the fall of arterial  $P_{O_2}$  that would be necessary to effect a fall of end-capillary  $P_{O_2}$  in normal blood equivalent to the fall due to acetazolamide.

data, the cerebral end-capillary pH after acetazolamide should be 7.46;  $P_{O_2}$  at the pH is 31.6 mm. Hg. Thus, the expected fall in end-capillary  $P_{O_2}$  due to acetazolamide is 35.7–31.6, or 4.1 mm. Hg. Superficially, this seems to be a minor fall in  $P_{O_2}$ . Calculations along the  $O_2$  dissociation curve, however, indicate that a fall of 33 mm. Hg in arterial  $P_{O_2}$  (from  $P_{O_2}$  90 mm. Hg) would be needed in the normal state to produce an equivalent fall in end-capillary  $P_{O_2}$ . Such a fall in arterial  $P_{O_2}$  is known to produce an increase in CBF.<sup>13</sup>

Our data show that tissue hypoxia does not occur after acetazolamide and thus cannot account for either the metabolic acidosis of ECF or the increase in CBF.

The cerebral vasodilation that follows acetazolamide administration may be related to the observed extracellular metabolic acidosis (table 1), or to a hypothetical intracellular carbonic acidosis. The latter possibility is at this writing entirely speculative: usually it is assumed that  $CO_2$  produced by decarboxylation in the mitochondria is in the gaseous form,  $CO_2$ , rather than the hydrated form,  $H_2CO_3$ . The unexplained high concentration of carbonic anhydrase in brain, and the rise in brain  $HCO_3^-$  concentration reported by Kjallquist and Siesjo<sup>19</sup> after acetazolamide are more consistent with the alternative that aerobic metabolism forms  $H_2CO_3$ , or  $H^+$  and  $HCO_3^-$ . If

so, acetazolamide would increase the intracellular concentration of carbonic acid,  $H^+$  and  $HCO_3^-$ .  $H^+$  may diffuse out more readily than  $HCO_3^-$ , acidifying the ECF. Also, it has been suggested by McDowell (personal communication) that the final common site for the effect of  $H^+$  on cerebral vessels may be intracellular rather than extracellular. Acetazolamide might act directly to acidify the interior of the muscle cell.

Since CBF was not measured directly in our experiments, CBF was expressed in terms of flow per unit  $O_2$  consumption ( $\dot{Q}/V_{O_2}$ ). Acetazolamide does not change  $V_{O_2}$  in man in doses equivalent to those used in our experiments,<sup>3</sup> so that  $\dot{Q}/V_{O_2}$  serves as an index of changes in CBF due to acetazolamide. Even in the face of a changing  $V_{O_2}$ , however, we feel that CBF per unit  $O_2$  consumption serves as a useful concept in evaluating CBF in terms of the demand for  $O_2$  by the tissues (e.g., a high  $\dot{Q}/V_{O_2}$  value will be associated with red venous blood in the "luxury-perfusion" syndrome as it was introduced by Lassen<sup>20</sup>).

Barbiturates reduce cerebral oxygen consumption.<sup>21</sup> pH has an effect on distribution such that acidosis drives barbiturates into lipids and deepens anesthesia. The pH in our experiments at any constant  $P_{CO_2}$  fell approximately 0.08 unit, and we might therefore attribute some of the observed effect on tissue and venous  $P_{O_2}$  to deepening anesthesia. This effect must be small, since the barbiturate level was decreasing with time.

The sagittal sinus blood in the dog may contain non-cerebral venous blood.<sup>22</sup> The relative contributions of cerebral and extracranial tissues cannot be assessed, nor can we assume that the decreased arteriovenous oxygen difference after acetazolamide accurately reflects cerebral flow. That cerebral flow does increase is supported by the four dogs in which Krypton clearance was determined over the exposed brain (table 1), and by the evidence cited in references 1-5.

In our data, CBF and  $P_{T_{O_2}}$  changes due to acetazolamide were plotted against  $P_{a_{CO_2}}$ . Although blood samples were analyzed for  $P_{CO_2}$  and pH immediately after sampling, there is evidence that the values measured in an *in vitro* electrode system do not represent *in vivo*

values after acetazolamide. Enough time elapses between the drawing of blood samples and analyzing them in the electrodes to allow for completion of the uncatalyzed hydration or dehydration of  $CO_2$ . The uncatalyzed reaction *in vivo* is incomplete during the circulation time from lung to brain, or from brain to lung. Brzezinski and his collaborators<sup>24</sup> pointed to this phenomenon that results in an overestimation of the true  $P_{a_{CO_2}}$  (underestimation of  $p_aH$ ) and underestimation of venous  $P_{CO_2}$  (overestimation of  $p_vH$ ). Plotting the changes in CBF or  $P_{T_{O_2}}$  against *in vitro* (i.e., measured)  $P_{CO_2}$ , as we have done, shifts the post-acetazolamide lines to a higher  $P_{a_{CO_2}}$  than exists *in vivo*. This means that, at constant  $P_{a_{CO_2}}$  values of 30, 40, and 50 mm. Hg, the increase in CBF and  $P_{T_{O_2}}$  that is due to acetazolamide is even larger *in vivo* than would be indicated from our data.

### Conclusions

These experiments failed to disclose any evidence of tissue hypoxia after acetazolamide. The fall of  $P_{O_2}$  predicted from blocking the Bohr effect in red blood cells appears to be overwhelmed by a rise of  $P_{O_2}$  due to increased flow. It follows that the increase in flow and the extracellular fluid acidosis are in no way due to hypoxia. The cause of these changes remains unidentified. We also conclude that the use of acetazolamide in association with artificial ventilation in the treatment of chronic hypercapnia appears justified.

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