Interrelations of the blood-aqueous potential and acetazolamide in the rabbit

James E. Miller

The intravenous administration of 100 mg. per kilogram of acetazolamide produced a 0.9 mv. abrupt fall in the 7 mv. resting potential, and was followed by a 1.4 mv. rise that persisted for more than 20 minutes. This alteration was not influenced by the previous injection (50 minutes) of 100 μg per kilogram of strophanthin K or nephrectomy (18 hours). One hundred milligrams per kilogram of ammonium chloride produced a 3.7 mv. rise in 37 seconds that lasted 25 minutes. If this injection was followed by acetazolamide in 2 minutes, the changes from the carbonic anhydrase inhibition could be noted, but the rise in positivity was prolonged for 37 minutes. The administration of 225 mg. per kilogram of 0.6M sodium bicarbonate lowered the gradient 1.5 mv. with a return to baseline at 18 minutes. An infusion of 535 mg. per kilogram for 90 minutes, however, did not alter the gradient or the response to acetazolamide in spite of the plasma bicarbonate being elevated to 29 mg. per cent. Sodium fluoroacetate (0.25 mg. per kilogram) reduced the potential 1.8 mv., and the increase of positivity due to acetazolamide was maintained for the duration of the experiment in 5 of the 6 rabbits.

It has been noted previously that a 7 mv. resting potential exists between the blood and aqueous, with the aqueous positive. This potential was altered by the intravenous administration of acetazolamide, which produced an abrupt 0.9 mv. fall followed by a 1.4 mv. rise that decreased over 20 minutes (Fig. 1).

Further investigation has shown that it is possible to influence the effect of acetazolamide by alterations in the acid-base balance. In addition, other metabolic inhibitors, such as fluoroacetate or strophanthin, were found to alter the potential gradient between aqueous and blood.

Method

The preparation of the rabbits and the recording system have been described previously. All of the agents, with the exception of sodium bicarbonate (NaHCO₃), were administered via the ear vein in less than 1 minute through a polyethylene cannula with an attached syringe. In the NaHCO₃ infusion experiment, a Harvard Infusion Pump was used to deliver 0.4 ml. per minute, and the anterior chamber electrode was inserted after completion of the injection. In all other determinations the potential was recorded prior to the injection. One hundred milligrams per kilogram of sodium acetazolamide (30 mg. per milliliter) was given in all experiments. In the group with nephrectomies, both kidneys were removed by lumbar incisions 18 hours prior to the determination.

Results

Strophanthin K. In 6 animals 100 μg of strophanthin K. (4.0 ml.) per kilogram was administered following the insertion
ACETAZOLAMIDE
Dosage: 100mg/kg.

NH₄ CI (100mg/kg)

Fig. 1. A 0.9 mv. decrease in potential occurred 25 seconds after administration of acetazolamide followed by a 1.4 mv. increase at 4 minutes.

STROPHANTHIN K
Acetazolamide (100mg/kg)

Stroph.

Acet.

Fig. 2. The administration of strophanthin K was accompanied by a 1.1 mv. decrease over a 30 minute interval. The effects produced by acetazolamide were not altered.

of the electrode into the anterior chamber, and a continuous recording was made for 50 minutes. The average resting potential was 5.8 mv. (standard deviation [S.D.] 1.6). In 4 of the 6 animals a gradual decline of 1.1 mv. (S.D. 0.5) was observed. In the remaining 2 animals there was no change from the preinjection potential.

Following acetazolamide, the usual occurrences were noted in all rabbits. The fall measured 1.2 mv. (S.D. 0.3) and the rise 1.5 mv. (S.D. 0.5) with the time of maximum rise averaging 4.6 minutes (Fig. 2).

In all animals the intraocular pressure decrease following strophanthin was measured by Schiotz tonometer. Preinjection tension determinations were 3.1 scale units (S.D. 0.5) and 7.8 scale units (S.D. 1.1) after strophanthin. In 3 animals a further 2.3 scale-unit decline in pressure was measured after acetazolamide. One of the remainder had a 2.0 scale-unit rise in pressure, and the other 2 did not change.

Nephrectomy. Nephrectomy 18 hours prior to the experiments did not change the potential or its response to acetazolamide in 6 animals. The preinjection potential gradient was 7.4 mv. (S.D. 1.6). A 1.1 mv. fall (S.D. 3.5) was noted 21 seconds after the administration of acetazolamide, and the subsequent rise was 1.5 mv. (S.D. 0.5). This occurred 4.6 minutes after the injection. The response was almost identical with that in the normal rabbit.

Ammonium chloride. Acidosis was induced in 6 rabbits by the administration of 100 mg per kilogram of a 3 per cent ammonium chloride solution (30 mg. per milliliter) injected intravenously over a 1 minute interval. There was a 3.7 mv. rise (S.D. 0.7) from the 2.8 mv. base line (S.D. 1.3) which occurred 37 seconds after the injection. The mean return to the base line occurred in 25 minutes (Fig. 3).

In 6 additional animals acetazolamide was given 2 minutes after the injection of ammonium chloride. The alterations caused by acetazolamide appeared to be superimposed upon the ammonium chloride response. However, the rise in positivity was prolonged for an additional 12 minutes (Fig. 4).

The intraocular tension was determined in the undisturbed eye by a Schiotz tonometer prior to the injection of ammonium chloride. The mean value was 4.6 scale units with a 5.5 Gm. weight. Thirty minutes after the injection, it was 10.8 scale units. In the animals given the combination of ammonium chloride and acetazolamide, the preinjection tension was 3.6
scale units and the postinjection tension 8.5 scale units.

It appeared that both ammonium chloride and acetazolamide increased the positivity of the anterior chamber with reference to blood and lowered intraocular tension. The combination of the two compounds prolonged the increase in potential but was not additive.

**Sodium bicarbonate.** Transient alkalosis was produced in 6 animals by giving 225 mg per kilogram of 0.6M sodium bicarbonate (50 mg per milliliter). Before injection the potential was 4.8 mV. A 1.5 mV. fall (S.D. 1.7) occurred in 36 seconds and lasted 18 minutes (Fig. 5). Schiotz determinations with 5.5 Gm. weight averaged 3.0 scale units before the experiment and 5.8 scale units after.

In 14 animals 225 mg per kilogram of 0.6M sodium bicarbonate was infused at the rate of 0.4 ml per minute. At the completion of the infusion the blood-aqueous potential was found to average 6.8 mV. (S.D. 1.4). Acetazolamide produced a 1.4 mV. decrease (S.D. 0.5) and a 1.2 mV. rise (S.D. 0.5). The increase in the potential gradient lasted for an average of 13.6 minutes.

The plasma pH and sodium bicarbonate were determined in 7 of the infused animals; the pH measured 7.71 at 25° C. (S.D. 0.1), and the plasma bicarbonate 29.4 mg per cent (S.D. 2.0). Schiotz determinations with a 5.5 Gm. weight averaged 2.9 scale units before infusion, 3.5 after infusion, and 5.5 after acetazolamide.

Alkalosis did not block the effect of acetazolamide, either by its alteration of the blood-aqueous potential or decrease in intraocular tension.

**E. Sodium fluoroacetate.** Sodium fluoroacetate at a dose of 0.25 mg per kilogram (0.5 ml.) was injected and the potential was recorded for 45 minutes. A gradual decline of 1.8 mV. (S.D. 0.2) was observed over the 45 minute interval. Following acetazolamide, the response was altered in that the increase in positivity of 2.3 mV. was maintained for the remainder of the experiment in 5 of 6 rabbits (Fig. 6). The initial fall did not appear to be altered in that it measured 1.4 mV. (S.D. 0.36). Sodium fluoroacetate decreased the normal blood-aqueous potential and altered the response to acetazolamide by prolonging the increase in positivity.

**Discussion**

It appears that the resting potential of 7 mV. between blood and aqueous is partially dependent upon sodium-potassium flux...
since there was a decrease of 1.1 mv. in
the potential following inhibition by stro-
phanthin K. This was similar to a 4.4 mv.
decline obtained when a higher local con-
centration of strophanthin G was adminis-
tered through the lingual artery. With the
latter technique a 60 per cent decrease in
aqueous flow was also measured. The po-
tential changes following administration
of acetazolamide were not altered by
strophanthin, indicating its independence
from sodium-potassium interchange. Thus,
it appears that the resting potential and
the alterations produced by acetazolamide
are related to aqueous formation, but in-
volve either separate steps in the same
process or separate transport systems.

The most prevalent hypothesis of the
action of acetazolamide in reducing aque-
ous secretion seems to be its influence
upon intracellular buffering capacity. By
its inhibition of carbonic anhydrase, the
production of bicarbonate ions is decreased
and leads to a metabolic acidosis. This
decrease is at least partially a result of its
local action on the eye since intracarotid
infusion of acetazolamide has been noted
to lower intraocular pressure initially on
the infused side. There was further sug-
gestion that the action of acetazolamide
occurred on a local basis by the normal
potential pattern obtained in animals after
nephrectomy.

A similar effect to acetazolamide was
produced on aqueous secretion by systemic
acidosis. However, the total effect of the
agent may be due to a combination of both
systemic and metabolic acidosis since the
blood pH was found to decrease 0.1 of a
unit 5 minutes after the injection of 100
mg. per kilogram of acetazolamide. This
interval coincides with the period of max-
imum positivity observed in the potential
measurements.

An increase in positivity also follows the
administration of acidifying salts, such as
ammonium chloride. The combination of
ammonium chloride and acetazolamide
maintained the increase in potential over
a longer interval than either alone, which
suggests a longer interval of intra- and
extracellular acidosis.

A decrease in the potential gradient ac-
companied transient alkalosis with a rela-
tively rapid recovery. Thus, the action of
acetazolamide on the potential gradient
between anterior aqueous and blood may
be explained on the combination of alkal-
osis and acidosis. The pH of the sodium
acetazolamide solution measured 9, and
produced a momentary alkalosis followed
by acidosis. The alkalosis decreased the
electrical gradient momentarily and was
immediately followed by carbonic anhy-
drase inhibition with resulting systemic
acidosis.

It has been observed that systemic al-
kalosis blocks the action of acetazolamide
upon aqueous secretion. The infusion of
sodium bicarbonate did not alter the po-
tential changes produced by acetazolamide.
However, a pressure decrease was obtained
in the control eye following acetazolamide.
Thus, under the conditions of these ex-
periments, the reversal of acetazolamide
effects by alkalosis was not verified either
by tension or by potential measurements.

The change seen after the injection of
fluoroacetate is similar to that of strophan-
thin in that the resting potential was
reduced, with the initial phase of the
acetazolamide cycle maintained. The con-
tinuation of the increase in positivity fol-
lowing carbonic anhydrase inhibition was
not attributed to systemic acidosis since
measurements have indicated that the de-
crease in plasma pH following acetazo-
lamide was either blocked or delayed. One
possible explanation for the prolongation
of positivity may be the production of met-
abolic acidosis without systemic changes.
This leads to the accumulation of acid
radicals within the eye so that a diffusion
current is maintained between blood and
aqueous.

REFERENCES
1. Miller, J. E.: Alterations of the blood-aqueous
potentials in the rabbit, Invest. Ophtalm. 1:
59, 1962.
2. Cole, D. F.: Electrochemical changes asso-