# Bicarbonate Ion Transport: A Mechanism for the Acidification of Urine in the Turtle

Abstract. *The uricotelic turtle* Pseudemys scripta *acidifies the urine to a* p*H as low as 4 in the urinary bladder. Data in this report show that the mechanism of acidification in this bladder is the transport of bicarbonate ion from lumen to serosa, and that the temperature to which the turtles are adapted prior to the in vitro experiment largely determines the direction of the transmural carbon dioxide gradient observed. This temperature effect also serves to reconcile apparently disparate data that were previously reported. A new technique for the direct determination of the partial pressure of carbon dioxide was employed.*

In principle, the *p*H and bicarbonate ion concentration of any body fluid could be regulated equally well by transporting either $H^+$ or $HCO_3^-$ into or out of the fluid as required, provided the controlled *p*H is between 4 and 8. Acidifying a fluid by transporting $H^+$ into it has many features in common with acidifying a fluid by absorbing $HCO_3^-$ from it. These properties are equally compatible with either mechanism of acidification. However, since the formation of gastric acid by $H^+$ secretion is well established and no example of acidification by $HCO_3^-$ absorption has been convincingly demonstrated, findings consistent with $H^+$ secretion are often taken as proof of $H^+$ secretion (*1*).

To determine the mechanism of acidification with certainty, it is necessary to determine the direction in which the reaction

$$CO_2 + H_2O \rightleftharpoons \hat{H}_2CO_3 \rightleftharpoons \hat{H}^+ + HCO_3^- \quad (1)$$

is being driven in the fluid being acidified. Once the direction of this reaction is known, the mechanism of acidification is uniquely determined. Net addition of $H^+$, by any mechanism, will drive the reaction to the left. Net absorption of $HCO_3^-$, by any mechanism, will drive the reaction to the right.

One way of determining the direction of this reaction is by the $CO_2$ gradient method, which is based on the following postulates: (i) in the steady state, the carbon dioxide pressure, $P_{CO_2}$, of a fluid surrounded by a metabolizing tissue will come into concentration equilibrium with the $P_{CO_2}$ of the cells facing the lumen, and (ii) the $P_{CO_2}$ of the cells of a metabolizing tissue submerged in a fluid with a fixed $P_{CO_2}$ will become greater than that of the external bathing fluid so that metabolically produced $CO_2$ can escape from the tissue by diffusion (*1*). Therefore, in such a system the $P_{CO_2}$ of the lumen fluid will be greater than that of the external fluid in the absence of an acidifying mechanism.

If the lumen fluid contains $HCO_3^-$ and is acidified by secretion of $H^+$ into the lumen, reaction 1 will be driven to the left and the $P_{CO_2}$ of the lumen will increase further. However, if the lumen fluid contains $HCO_3^-$ and is acidified by transporting $HCO_3^-$ out of the lumen, reaction 1 will be driven to the right and luminal $P_{CO_2}$ will decrease. If the $HCO_3^-$ transport rate is sufficiently rapid to cause the rate of hydration of $CO_2$ in the lumen fluid to exceed the rate of diffusion of metabolic $CO_2$ into the lumen, then the $P_{CO_2}$ of the lumen fluid could become less than that of the external bathing fluid. The $P_{CO_2}$ of the lumen fluid would then be necessarily less than that of the tissue, which is a source of $CO_2$ (*2*). Such a finding could only be explained by a transport system moving $HCO_3^-$ from lumen to serosa. There are other possible mechanisms for lowering luminal $P_{CO_2}$ in such a system, but they all inevitably lead to an increase in luminal *p*H as long as $HCO_3^-$ is present in the luminal fluid.

Therefore, if the luminal $P_{CO_2}$ were found to be greater than that of the external fluid, the results would be equivocal and would be consistent with hydrogen secretion, or a low rate of $HCO_3^-$ absorption, or no acidifying mechanism at all. However, the finding that the $P_{CO_2}$ of the acidified lumen fluid is less than that of the external bathing fluid in such a system would be the unique result of $HCO_3^-$ transport from lumen to serosa.

The first investigation of the mechanism of acidification of the lumen fluid by the urinary bladder of the turtle was performed with experiments based on the $CO_2$ gradient principle (*3*). The results supported the idea that the mechanism was $HCO_3^-$ ion transport. Later investigators, using the same principle but different analytical techniques, were unable to confirm the earlier results (*4*). Because of this conflict, I decided to reinvestigate the matter, using experiments designed on the same principle but employing a third and independent analytical technique. Since Hernandez and Coulson (*5*) have reported that reptilian renal function deteriorates when the animals are subjected to low temperatures, animals adapted to various temperatures were included in the experiments.

In the work reported here turtles were housed for two or more weeks at 21°, 26°, or 32°C. At 21°C the turtles were sluggish, did not eat for 4 weeks or more, and had a urine *p*H of 6.8 to 7.2. At 26°C the turtles were active, ate every 2 weeks, and the *p*H of the urine was 6.5 to 7.0. At 32°C the turtles were aggressive, ate every 4 days, and the urine *p*H was 4.1 to 6.6. The temperatures at which the animals were housed had a much greater influence on the results of the experiments in vitro than did the temperature at which the experiments were carried out. When bladders taken from 21°C turtles were incubated at 21°, 26°, or 32°C, the luminal $CO_2$ concentration remained above the serosal $CO_2$ concentration.

The bladders were tied over the end of the side arm of a Van Slyke apparatus, serosal side out. They were filled with 4 ml of an isotonic, gas-equilibrated solution (NaCl, 95 m*M* + $HCO_3^-$, 5.0 m*M*, *p*H 6.5) and submerged in 500 ml of a complete Ringer solution including $HCO_3^-$ (15 m*M*, *p*H 7.0), which was continuously equilibrated with the same gas (7 percent $CO_2$ and 93 percent $O_2$) at 26°C. The incubation period was 4 to 5

Table 1. Data used to determine the mechanism of acidification of the lumen fluid by turtle urinary bladder. Shown are the temperature at which each group of turtles was housed; the incubation period required for the bladder to reduce the luminal *p*H by approximately 0.6; the decrease in the luminal $HCO_3^-$ concentration during the incubation; the change in the luminal concentration of free $CO_2$ with time; and the differences (mucosal minus serosal) between the luminal and serosal concentrations of free $CO_2$ at the end of the incubation period. The *P* values were obtained by comparing mean transmural gradients to 0.0054 ± 0.025 in an unpaired Student's *t*-test. Square brackets denote concentration.

| Housing temperature (°C) | *N* | Incubation period (hours) | Mean change in mucosal fluid parameters with time | | | Mean final transmural difference $[CO_2]_m - [CO_2]_s$ (m*M*) |
|---|---|---|---|---|---|---|
| | | | *p*H | $HCO_3^-$ (m*M*) | $CO_2$ (m*M*) | |
| 21 | 6 | 5.0 | −0.66 | −3.16 | 0.34 | 0.346 ± 0.054 ($P < .001$) |
| 26 | 11 | 4.5 | −0.60 | −2.62 | 0.08 | 0.104 ± 0.027 ($P < .025$) |
| 32 | 11 | 4.0 | −0.63 | −3.65 | −0.13 | −0.127 ± 0.025 ($P < .005$) |



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hours. All bladders were allowed to acidify the lumen fluid to about the same degree. Mucosal samples for determination of the free dissolved $CO_2$ in the lumen fluid were drawn directly into the Van Slyke apparatus through the side arm. Manometric measurements were made at constant gas volume. Pressure $P_1$ was obtained after degassing the native sample, $P_2$ after degassing the acidified sample, and $P_3$ after degassing the alkalinized sample. Free $CO_2$ was determined from the difference $P_1 - P_3$ and $HCO_3^-$ from $P_2 - P_1$. The sample volume was determined from the weight of displaced mercury. Lumen fluid *p*H was determined from a second anaerobic sample.

To test the analytical technique, portions of 13 final samples were paired with portions of 0.1*N* $H_2SO_4$ and equilibrated with the same gas. The concentrations of dissolved free $CO_2$ in the gas-equilibrated $H_2SO_4$ solution and mucosal fluid were determined as above and compared. The mean difference between them was 0.0054 ± 0.025 m*M*.

Dissolved $CO_2$ cannot be measured directly in the serosal fluid because the fluid contains buffers other than $HCO_3^-$. In each experiment serosal dissolved $CO_2$ was determined from total $CO_2$ and *p*H measurements (*pK* 6.19) and from measured $CO_2$ dissolved in 0.1*N* $H_2SO_4$ coequilibrated with the same gas. Whenever there was a discrepancy in these two values for the free dissolved $CO_2$ concentration, the value obtained in the coequilibrated acid was assumed to be correct. In 13 such paired measurements the mean difference between the concentrations of dissolved $CO_2$ determined in the acid and in the Ringer solution was 0.016 ± 0.014 m*M*. These control data on mucosal, serosal, and sulfuric acid solutions show that the analytical techniques are valid.

At the beginning of the incubation, the *p*H, dissolved $CO_2$, and $HCO_3^-$ were determined in the mucosal and serosal fluid. At the end of the incubation period one to four sets of similar measurements were made, depending on the availability of the lumen fluid.

Table 1 shows the number of turtles housed at each of the three different temperatures together with the mean change in the $CO_2$ and $HCO_3^-$ concentrations and *p*H of the lumen fluid during the incubation in vitro of the bladders from each group of turtles. In each case the *p*H of the lumen fluid fell by 0.6 and the luminal $HCO_3^-$ was reduced by about 60 percent. While all incubations were at 26°C, the bladders from the 32°C turtles acidified the lumen fluid most rapidly. Since the initial free $CO_2$ of the lumen fluid approximated that of the serosal fluid by design, the temporal changes in luminal $CO_2$ reflect the final transmural difference in $CO_2$ in magnitude and direction. The critical data, the mean final transmural differences in free $CO_2$ (mucosal value minus serosal value), are given along with their standard errors and *P* values. It can be seen that the final luminal $CO_2$ concentration decreases as the bladder acidifies the lumen fluid more rapidly. The bladders from 32°C turtles caused the hydration of luminal $CO_2$ at a rate sufficient for the hydration to outrun the inward diffusion of metabolic $CO_2$ and drive the luminal $CO_2$ concentration below that of the serosal fluid. Therefore, the mechanism of acidification must be the transport of $HCO_3^-$ ion from lumen to serosa. This is one example of a bicarbonate ion transport system capable of regulating the *p*H of a body fluid in the *p*H range 4 to 8. The temperatures for housing the turtles and incubating the bladders were chosen arbitrarily and may not be optimal values.

The temperature at which the turtles were housed was not taken into account in previous work on this problem. The data presented here show that workers housing turtles at different temperatures but doing otherwise identical experiments would be expected to get different results, and that the finding that the luminal $P_{CO_2}$ is greater than the serosal $P_{CO_2}$ should be interpreted as evidence for $H^+$ secretion.

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**References and Notes**

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## Frog Perspective on the Morphological Difference Between Humans and Chimpanzees

Abstract. *The body shapes of humans and chimpanzees were compared quantitatively by criteria chosen for their capacity to discriminate well among the body shapes of frogs. By these criteria, the difference in body shape between humans and chimpanzees was found to be greater than that between the most dissimilar pairs of frogs examined—that is, frogs classified in separate taxonomic suborders. Even though the morphological difference between the two primates is large by frog standards, the biochemical differences between the structural genes of these two species are small. The results of this study give quantitative support to the proposal that morphological evolution and biochemical evolution in structural genes can proceed at independent rates.*

Biochemistry and morphology give us contrasting views of the difference between humans and chimpanzees. Biochemical comparisons made with proteins and nucleic acids indicate that humans are remarkably similar to chimpanzees at the gene level (*1*). The structural genes of this pair of species are more similar than the structural genes of most pairs of species within a genus, regardless of whether the species compared are vertebrates or invertebrates (*1*). This biochemical picture, however, contrasts with that provided by morphologists who assign chimpanzees and humans not just to separate species but to separate taxonomic families (*2*). Thus, the morphological difference between these two species appears large, whereas the biochemical difference is small.

King and Wilson (*1*) inferred from this and other evidence that structural gene evolution and morphological evolution may proceed at independent rates. Some biologists, however, have been reluctant to agree with King and Wilson that there really is a contrast between the morphological and biochemical results of evaluating the difference between chimpanzee and human (*3*). Although these biologists are aware of the quantitative and objective nature of the biochemical comparisons, they are also aware that the chimpanzee-human morphological difference has never been compared quantitatively with the morphological differences existing among other species. This lack of confidence in the morphologists' judgment that the chimpanzee-human difference is as big as that among the families of other animals is illustrated by Merrell (*3*). He stated, in essence, that if a nonmammalian creature were to classify animals on the basis of morphol-


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