Intracellular CO_2 Tension: Practice and Theory*

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I have been asked to discuss methods of measuring intracellular P_{CO_2} and to discuss the physiology and biochemistry of intracellular CO_2 tensions. Actually, I am in a relatively fortunate position in that my difficulties in discussing the methodology of intracellular P_{CO_2} measurements are considerably less than those which face my two colleagues. The reason for this becomes apparent if one analyzes the following quantitative relationships.

Gas Exchange

Let us consider a simple model of a cell and its blood supply. I have arbitrarily divided this blood supply into an inspiratory portion at the arterial end of the capillary and a mixed expiratory portion at the venous end of the capillary. To simplify analysis of the relationship between blood gas tensions and intracellular gas tensions, I have made certain assumptions with respect to this model.

1. It is assumed that the fluid which circulates through the vascular channel is plasma and plasma alone. It is assumed that there are no red cells or hemoglobin contained in this plasma. Although one can derive an expression for cellular gas exchange in the presence of hemoglobin (capable of combining with O_2 and CO_2), it is a more complicated equation, and, therefore, analysis is based on hemoglobin-free plasma. This is not an unreasonable assumption, for there are living systems which receive all of their O_2 supply from plasma. For example, tunicates apparently derive required O_2 solely from plasma despite the fact that this species has "green" blood cells containing a vanadium pigment which does not function as an O_2 carrier. An Arctic fish studied by Ruud lacks hemoglobin. Other animals, like some teleost fish, have rather low hematocrits, averaging between 6% and 9%. The assumption that O_2 carriage by hemoglobin is absent is, therefore, not an unreasonable one.

2. It is assumed that gas exchange between cell and plasma occurs entirely by passive diffusion. No special mechanisms for either O_2 or CO_2 transport are involved.

3. It is assumed that the rate of blood flow through the capillary is slow enough to allow establishment of complete gas tension equilibrium between cell and plasma at the venous end of the cell.

A relationship between mean O_2 and CO_2 tensions in the cell can now be obtained as follows. The amount of CO_2 given off by the cell as the plasma flows from the arterial to the venous end of the capillary is equal to the rate of plasma flow times the solubility coefficient of CO_2, α, (which, as you know, relates content of CO_2 to tension of CO_2) times the difference in partial pressures at the expiratory and the inspiratory ends of the vessel. Expiratory and inspiratory P_{CO_2} may be regarded as equal to the plasma P_{CO_2} at the

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arterial and venous ends of the vessel, respectively, in accordance with the third assumption stated above. In a similar fashion we can quantitate the oxygen uptake for the cell as being equal to the flow times the solubility coefficient for oxygen times the difference in Po_2 in the plasma at the arterial and venous ends of the capillary. Dividing one equation by the other gives the respiratory exchange ratio (RQ). The flows cancel out, and one is left with the ratio of solubility coefficients for CO_2 and O_2 times the respiratory quotient (R). This equation may now be solved for the mean plasma Po_2 at the expiratory side of the cell. The result is:

$$P\bar{E}_{O_2} = P_{I_{O_2}} - \frac{1}{R} \times \frac{\alpha\, CO_2}{\alpha\, O_2} \cdot (P\bar{E}_{CO_2} - P_{I_{CO_2}}) \qquad (1)$$

Inserting reasonable values for these parameters, one can then solve for the variable that we are interested in, namely $P\bar{E}_{CO_2}$. The mean $P\bar{E}_{O_2}$ is unknown. Its absolute value is not important, however, for the calculation of the CO_2 tension inside of the cell. We shall assume a high value, say, 40 mm Hg. By definition, $P_{I_{O_2}}$ is equal to the P_{O_2} of arterial plasma, namely, 90 mm Hg. R has a value very near to "one." The solubility for CO_2 and O_2 ratio depends upon temperature and upon ionic strength, but under the present conditions (mammalian temperature and ionic strength) this ratio equals about 20/1. The partial pressure of CO_2 in the inspired fluid, $P_{I_{CO_2}}$, is equal to the arterial CO_2 tension, which is 40 mm Hg. One may now examine the effects of changes of these independent variables on the value of $P\bar{E}_{CO_2}$, the dependent variable. One can show that, lowering $P\bar{E}_{O_2}$ from 40 mm Hg to 1 mm Hg, the $P\bar{E}_{CO_2}$ (= intracellular P_{CO_2} by definition) increases from about 42.5 mm Hg to approximately 45 mm Hg. Thus, for very large changes of $P\bar{E}_{O_2}$, the effect on $P\bar{E}_{CO_2}$ is very small. A change of RQ, from its maximal value of "one" in the steady state to a minimal value of around .7, changes the value of intracellular P_{CO_2} by only 0.5 mm Hg. The ratio of gas solubilities is relatively constant. Therefore, despite a wide spectrum of changes in the relationship of all of the other factors, there is a very close relationship between the P_{CO_2} of venous blood and $P\bar{E}_{CO_2}$, i.e., the P_{CO_2} inside the cell. In other words, CO_2 is so soluble and its diffusibility so high in comparison with oxygen that measurements of P_{CO_2} in venous blood must closely approach the mean P_{CO_2} inside the cell. If one can obtain an approximate value for the P_{CO_2} in a given organ, that is to say, if one can measure the P_{CO_2} of the venous blood which drains that organ, one can approximate the value of intracellular P_{CO_2}. This approximation is likewise acceptable for calculation of whole body intracellular P_{CO_2} if the calculations are based on measurements of mixed venous blood P_{CO_2}. Since venous plasma is a homogeneous fluid in which measurements can be made with reasonable accuracy, one can closely approximate a value for mean intracellular P_{CO_2}.

I would like to contrast this situation with that faced by both of my colleagues in this seminar. Dr. Jöbsis has implied that, from the standpoint of cellular metabolic processes, "mean" oxygen tension probably signifies little, because one needs to know the values of the O_2 tension at those sites in the cell where the various O_2-consuming reactions are taking place. Precise analysis of the quantitative aspects of oxidative metabolism within the cell are very difficult.

There are two possible mechanisms by which O_2 enters from capillary blood into the cell. One possibility is that O_2 diffuses into the cell as a result of partial pressure difference between arterial capillary plasma and intracellular fluid. Those portions of the cell with the most extensive O_2 consumption receive the most O_2 because they are consuming it more rapidly, thereby producing a greater partial pressure difference, which in turn insures an adequate supply to the involved sites. If this is true, then there must be parts of the cell, for example, the cytoplasm, which have a partial pressure of O_2 close to that of arterial plasma, and other areas, such as the mitochondrion, that may have a partial pressure of O_2 of approximately 1 mm Hg. Although the concept of "mean O_2 tension" may be useful for some purposes, it has little validity in describing the nature of O_2 exchange at those intracellular sites where O_2 exchange occurs.

The second possibility to explain oxygen entry is that simple diffusion does not explain the transport of O_2 into the cell. Even under these circumstances, say, the existence of a special O_2 carrier, the bulk of evidence indicates that there is a spectrum of O_2 tensions within the cell.

The situation is as difficult when one deals with H^+ concentrations inside the cell. One may agree with Dr. Carter that H^+ inside the cell is in thermodynamic equilibrium with H^+ in extracellular fluid; or one may agree with others who take the viewpoint that thermodynamic equilibrium for H^+ exists in some cells, like the red cell, but that in other cells the mechanism for H^+ distribution is obscure. At any rate, it seems quite clear that the H^+ concentration inside the cell is different from that of plasma. The main problem in understanding H^+ relationships within the cell is the problem of H^+ concentrations at particular sites where H^+ is involved in modifying protein structure or reaction rates. It is interesting to note that an important issue in contemporary biochemistry involves the question of whether intramitochondrial pH is 2.0 or 6.0. At present such questions cannot be answered definitively.

It appears, then, that, in considering intracellular gas tensions, one is on more solid ground with the

very diffusible gas CO_2, because the differences between it inside cell water and venous blood cannot be large.

The P_{CO_2} in venous plasma depends upon, among other things, the amount of CO_2 produced and the amount of blood ventilating the cell. One of the important factors which determines the value of venous plasma P_{CO_2} is the level of inspired P_{CO_2}, which is, of course, the arterial P_{CO_2} level. The latter, in turn, is determined by the total CO_2 output of the animal or human subject and the alveolar ventilation. One must now raise the questions: What is it that sets the level at P_{CO_2} = 40 mm Hg in man living under normal circumstances? and Why is it that in certain animals the P_{CO_2} may be as low as 1 mm Hg? A survey of P_{CO_2} data obtained on a variety of animals is given in Table 1. For a bony fish, a shark and a tadpole, the P_{CO_2} values are 1.8 mm, 4.0 mm and 1.5 mm Hg, respectively. When the tadpole has developed into a frog, P_{CO_2} becomes approximately 20 mm Hg. The African lung fish, which, despite its name, is an air breather, has a P_{CO_2} of approximately 19 mm Hg. Turtles and seals have P_{CO_2}'s of 23 mm Hg and 42 mm Hg respectively, as compared to humans with a P_{CO_2} of 38 mm Hg. An analysis of such data reveals an apparent bimodal distribution of P_{CO_2} which operates in such a way that animals that exchange gas in an aquatic environment have very low CO_2 tensions in their body fluids, generally below 5 mm Hg. Animals that exchange gas with atmospheric air generally have CO_2 tensions above 10 mm Hg, sometimes as high as 40 or 50 mm Hg.

TABLE 1

P_{CO_2} Tensions In Vertebrates

I. Aquatic Gas Exchangers	mm Hg
Teleost	1.8
Elasmobranch	4.0
Tadpole	1.5
II. Aerial Gas Exchangers	
Lungfish	19.0
Turtle	23.0
Man	38.0
Seal	42.0
Frog	19.0

To explain this bimodal distribution of CO_2, one may use the gas exchange equation (1). Instead of applying the equation to a single cell, however, let us apply it to the whole organism, and let us assume for simplicity that $P_{I_{CO_2}}$ is equal to zero. Equation 1 may then be written as follows:

$$P_{\bar{E}_{CO_2}} = (P_{I_{O_2}} - P_{\bar{E}_{O_2}}) \frac{\alpha O_2}{\alpha CO_2} \times R \quad (2)$$

Assuming reasonable values applicable to: a) aquatic gas exchangers, and b) aerial gas exchangers (man), one finds:

a) $P_{\bar{E}_{CO_2}}$ = (150–100) × 1 × 1/35 = 1.4 mm Hg (18°C)

b) $P_{\bar{E}_{CO_2}}$ = (150–110) × 1 = 40 mm Hg (37°C)

For aerial gas exchangers the gas solubility ratio does not, of course, enter into the calculations.

It follows from this that the very low P_{CO_2} tensions found in aquatic gas exchangers is dictated by an obligatory requirement for O_2 and the ratio of the solubility of CO_2 to the solubility of O_2 in water.

Electrolyte, pH Relationships

Let us now consider the relationship between P_{CO_2}, HCO_3^-, and pH. This is expressed by the well-known Henderson-Hasselbach equation:

$$pH = pK' + \log \frac{[HCO_3^-]}{\alpha P_{I_{CO_2}}}$$

There is only a relatively narrow range of pH which is compatible with life. Therefore, for a fixed pH, $[HCO_3^-]$ must be high or low if P_{CO_2} is high or low. The highest $[HCO_3^-]$ found in aquatic animals with a low P_{CO_2} is approximately 9 to 10 mEq/1, as compared to man and other mammals with a relatively high P_{CO_2}. Here $[HCO_3^-]$ is, on the average, 25 mEq/1 and can be as high as 35 mEq/1 (Table 2). Thus, the bimodal distribution of P_{CO_2} referred to earlier is accompanied by a bimodal distribution of $[HCO_3^-]$.

Once the level of $[HCO_3^-]$ is determined, it becomes clear that the level of the other major extracellular anion, Cl^-, must be determined. Since electroneutrality must be maintained, aerial gas exchanging animals with a high $[HCO_3^-]$ must have a low $[Cl^-]$ in contrast to aquatic gas exchanging animals in which one finds a low $[HCO_3^-]$ and a high $[Cl^-]$. It therefore seems that the anion pattern of plasma in lower animals as well as in man is dependent on an obligatory requirement for O_2 and the form of gas exchange necessary to provide the O_2.

Let us now look at what the consequences of those relationships are on renal function. The kidneys are chiefly responsible for establishing and maintaining in plasma the proper $[HCO_3^-]$ and $[Cl^-]$, which are dictated by the law of electroneutrality. The renal tubule operates so that there is, more or less, an inverse relationship between the amount of $[Cl^-]$ and the amount of $[HCO_3^-]$ which is reabsorbed from the glomerular filtrate. Therefore, it appears that the renal pattern of conservation of the main anions depends, in the last analysis, on the

TABLE 2

Bicarbonate Concentrations in Body Fluids of Vertebrates

I. Aquatic Gas Exchangers	mEq/1
Teleost	4.0
Elasmobranch	7.0
Tadpole	6.0
II. Aerial Gas Exchangers	
Frog	12.0
Lungfish	12.0
Turtle	32.0
Man	25.0
Seal	27.0

mode of gas exchange and on an obligatory requirement for O_2.

One is tempted now to present certain biochemical features of the evolution of animals, as follows. The most primitive form of energy production is anaerobic glycolysis. Under these circumstances significant amounts of CO_2 are not produced, and the CO_2-[HCO_3^-] pair is not an important buffer system. As animals developed O_2-consuming pathways, they required O_2 for energy production to maintain life. A definite relationship between P_{CO_2} and P_{O_2} had to be established. Under some circumstances this led to a high P_{CO_2}, while other circumstances resulted in a low P_{CO_2} of body fluids. This, in turn, determined the electrolyte pattern of extracellular fluid and, presumably, intracellular fluid. It also determined specific patterns of renal function. It, thus, appears that the metabolic pathways that were so beautifully outlined by Dr. Jöbsis have an interesting general biological meaning. Many functions of animals which, a priori, seem to be isolated phenomena, depend on the simple fact that a given amount of O_2 is required for energy metabolism, and the pattern of these functions evolves as a consequence of the operation of the physical chemistry of gas exchange between animals and their environment.

Let us now consider CO_2 as an acid. A fruitful way to examine the effects of CO_2 on acid-base equilibria in animals is to analyze so-called whole-body, CO_2 titration curves. One exposes the animal to a range of P_{CO_2}'s and then determines the effect of these P_{CO_2}'s on the pH and [HCO_3^-] of arterial plasma. The range of P_{CO_2} changes in tensions in man and animals living at an ambient O_2 tension of 150 mm Hg is extensive. P_{CO_2} can vary from several millimeters of mercury in the shark to at least 100 mm Hg in man and dog.

Let us consider the quantitative aspects of pH changes in dog, man and shark. First of all, if one compares acute pH changes in dog obtained from the data of Schwartz, when P_{CO_2} is changed from 40 mm Hg to 140 mm Hg, with pH changes occurring "chronically," when dogs are placed into a CO_2-rich atmosphere for three weeks, one finds a smaller change of pH with changes in P_{CO_2}. In other words, within a given P_{CO_2} range, the acute increases of plasma in [HCO_3^-] are much smaller than changes that take place when the dog is chronically exposed to CO_2. It may be that the acute increase in [HCO_3^-] during the first 12- to 16-hour period takes place largely as a result of buffering of carbonic acid, especially by OH^- groups in intracellular fluid. Increase in [HCO_3^-] in animals exposed chronically to elevated P_{CO_2} not only reflects buffering but also reflects H^+ excretion by the kidneys. Exposure to high P_{CO_2}, either acutely or chronically, however, will lead to lower pH values in plasma. In other words, extracellular pH is not fully protected by an adequate generation of bicarbonate.

There are two possible general explanations of this finding. One explanation is that the ability of the animal to generate sufficient [HCO_3^-] to normalize extracellular pH is limited by either buffer capacity or renal function, or by both. This appears unlikely, since the [HCO_3^-] generated at high P_{CO_2} values would be adequate to normalize pH at intermediate P_{CO_2} values. The animal is clearly capable of generating a higher [HCO_3^-] than he does at intermediate P_{CO_2} values. Therefore, a functional limitation involving buffering or renal H^+ excretion does not appear likely.

The other possiblity is that the regulatory mechanisms involved are not geared to maintenance of a normal extracellular pH. This possibility requires emphasis since hypercapnic human subjects, unlike dogs, may maintain extracellular pH within normal limits until a relatively high P_{CO_2} is reached. To explain this fact, various theories have been presented. It has been suggested that such data are unreliable. This is unlikely, because similar findings have been reported by several independent investigators. The existence of a normal extracellular pH in the face of chronic hypercapnia is not unusual in man. The second theory that has been advanced to explain the data is that a normal or even high pH suggests the presence of superimposed disease. If a patient has a high P_{CO_2} and a normal pH, he may have developed independent metabolic alkalosis. The difficulty with this theory is that, frequently, one is unable to find clear-cut evidence of superimposed metabolic alkalosis. A third theory (our own) suggests that patients with chronic hypercapnia may regulate intracellular and not extracellular pH. Furthermore, it is suggested that intracellular regulation may be associated with low, normal or even high extracellular pH values.

Data were obtained by a young colleague of mine, Dr. Tushan, who studied a group of patients with moderate hypercapnia using the DMO technique to calculate whole-body intracellular pH and intracellular pH of muscle on biopsy samples. It was shown that the mean values for whole body intracellular pH and muscle pH are not significantly different from each other or from normal values, regardless of the level of extracellular pH. I should emphasize that this is not incontrovertible proof, but it is at least consistent with the theory that the regulatory mechanisms called upon when conditions of high P_{CO_2} exist are located in areas in the body which are not readily definable but are presumably intracellular in location.

I would now like to call your attention to the data from the shark, because the findings are dramatic and because I think they illustrate a rather important point. When the shark's P_{CO_2} is elevated from 4 mm Hg to 12 mm Hg, his extracel-

lular pH remains unchanged. The reason for this is that the animal generates bicarbonate extensively. For example, at P_{CO_2} = 12 mm Hg, the [HCO_3^-] is approximately 15 mEq/1. A two- to three-fold increase in P_{CO_2} results in an increase in [HCO_3^-], with maintenance of a normal extracellular pH. It is remarkable that this maintenance of extracellular pH is accomplished by intracellular buffering. No increase in H^+ secretion in urine or increased H^+ excretion by the gills is detectable. We are unable at present to explain this admirable performance of the shark. (It is possible that this remarkable degree of regulation occurs as a result of the characteristics of body buffering of H_2CO_3 in the low P_{CO_2} range and could also be observed in man if man could live under such conditions.) It is also possible that the shark has some special mechanisms, not found in man, for buffering the augmented H_2CO_3 resulting from an increase in inspired CO_2. Obviously more work is needed to find out which mechanism, or mechanisms, are involved.

I would now like to mention briefly the metabolic consequences of exposure to high and low P_{CO_2}. There are a number of reactions in man and other vertebrates in which CO_2 plays a role. To my knowledge there are in man no biochemical reactions in which CO_2 concentration is rate limiting. It should be emphasized, however, that an increase in P_{CO_2} leads to alterations of certain functions. For example, respiratory alkalosis damages brain function. Very high P_{CO_2} can induce seizures followed by narcosis.

Anaerobic Energy Metabolism

Stimulated by the lecture of Dr. Jöbsis on oxidative, energy-liberating pathways, I would like to conclude my presentation with a discussion of anaerobic energy production, which has not been discussed in this seminar.

There are at least four reasons why animals die when they undergo severe O_2 depletion. Dr. Jöbsis has already given one reason, namely, that, with loss of O_2-dependent reactions, energy production fails or ceases. Secondly, there is the loss, at least in mammals and in most vertebrates, of central nervous system integrity. There may be something about oxygen, aside from its ability to provide energy, that is necessary in vertebrate systems for the structural and functional integrity of the nervous system. Thirdly, animals suffering from oxygen depletion lose biosynthetic ability. In many biosynthetic reactions, oxygen acts as an obligatory oxidant for the synthesis of many important compounds. Fourthly, animals can die because of acidosis, which occurs as follows. As the oxygen supply becomes limited, the animal switches to anaerobic glycolysis, the end product of which is pyruvic acid. Pyruvate accumulates, as does lactate, its reduction product, along with other organic acids of the Krebs cycle. Thus, there is an accumulation of organic acids, which are proton donors, and the animal develops severe acidosis. This, combined with severe hypoxia, may become a limiting factor in survival.

Several years ago I inaugurated studies on pond turtles (*Pseudemys*). This species is able to dive for periods of several days. It was formerly believed that during diving the turtle respired by pulling water through the cloaca into two accessory bladders. I investigated this possibility by placing a plaster cast in the turtle's cloaca and blocking gas exchange through the buccal mucosa by taping the mouth. It turned out that there was no respiratory exchange under these circumstances, and the turtle survived under water for one to two weeks despite a total absence of external oxygen supply. Within a few hours the oxygen tensions in lung, in arterial and venous blood dropped to zero. The animal survived with O_2 tensions at essentially zero. The turtle was also able to survive in pure nitrogen. It likewise survived in the absence of cytochrome-dependent metabolism, as was demonstrated by blocking cytochrome c_3 with cyanide or by blocking cytochrome b with antimycin. Blood lactate levels rose from 1 mEq/1 to 40 to 60 mEq/1 over a period of seven to ten days, indicating that the major energy source was anaerobic glycolysis.

The precise mechanisms of anaerobic glycolysis under these circumstances is not known, but they may very well involve the pyruvate-lactate system. Pyruvate occupies a key position between anaerobic glycolysis and the aerobic pathway. Pyruvate can be interconvertibly reduced to lactate through the action of a group of isoenzymes which are collectively known as LDH (lactic dehydrogenase). In most vertebrates there are two basic kinds of LDH. There is an MLDH (muscle LDH) and an HLDH (heart LDH), the synthesis of each being under separate genetic control. The five isoenzymes which are found in most vertebrates represent the five possible permutations of these two polypeptides occurring as tetramers. Thus, there is an M_4, an M_3H_1, an M_2H_2, an M_1H_3, and an H_4.

MLDH is biochemically quite different from HLDH in a number of respects. According to Kaplan and his co-workers, MLDH, but not HDLH, is insensitive to inhibition with pyruvate ion. This means that if MLDH is dominant over HLDH, pyruvate is converted into lactate, possibly by supplying NAD, thus augmenting anaerobic glycolysis. On the other hand, if HLDH is dominant over MLDH, lactate will be converted into pyruvate, which is fed into the Krebs cycle and used in the oxidative metabolism. Thus, dominance of MLDH favors anaerobic glycolysis; dominance of HLDH stimulates oxidative metabolism. Although the role of the two forms of LDH in anaerobic glycolysis, as outlined here, is not fully established, there is good

evidence that the amount of MLDH or HLDH that is available, at least under some circumstances, is dependent on ambient O_2 tensions. Hypoxia is associated with high MLDH; aerobiosis with high HLDH levels. We have found that whole turtle homogenates and homogenates prepared from heart, brain, liver, or turtle serum have only one LDH, and preliminary work shows that this LDH is of the M type. This may be looked upon as a fortunate evolutionary development, because it is the presence of MLDH which, in part, makes it possible for the turtle to survive by anaerobic glycolysis without O_2 living. The question may now be raised: How does the turtle survive severe acidosis for days or weeks? The turtle has an anatomical compartment which is known as the coelomic cavity. It is equivalent to the peritoneal cavity in man, and the turtle may be likened to a patient suffering from liver cirrhosis in that the coelomic compartment has in it a volume of fluid which represents about 6% or 7% of total body weight. This fluid contains 100 to 120 mEq/1 of bicarbonate, as compared to plasma, which contains approximately 32 mEq/1. This compartment is also fairly permeable to lactate ion. The coelomic fluid, then, represents a relatively large reservoir or buffering bicarbonate solution, which provides for adequate protection against acidosis during diving. Indeed, one finds that, after diving, the bicarbonate concentration of the coelomic fluid decreases, while the lactate concentration increases.

DISCUSSION

Dr. Patterson: Dr. Robin is open for quizzing.

Dr. Jöbsis: As the lactate is freed by the body, it reacts with the very high concentration of bicarbonate here. Before that time there must have been an equilibrium. In other words, does the pH change vary greatly, or is CO_2 excreted by the animal?

Dr. Robin: No, you see he is diving. He cannot excrete CO_2, so the system is not as efficient as if he had open egress to air to get rid of the excess CO_2. On the other hand, in real life I imagine that pond turtles do not very often dive for as long as one to two weeks, and, therefore, the ability to come up for air and to get rid of CO_2 is present in life.

Dr. Jöbsis: What is the normal pH of the coelomic fluid?

Dr. Robin: Control values are $\sim$8.2. The fluid is like a solution of three-tenths molar Na^+ bicarbonate. During prolonged diving, the pH falls; lactate rises; bicarbonate decreases; and the P_{CO_2} rises sharply so that the animal has two sources of P_{CO_2}, the most important of which is due to the buffering of hydrogen ions by bicarbonate.

Dr. Regelson: It cannot only be that, though, because Sanford Siegal, exobiology researcher of Union Carbide, using the red-eared turtle for survival studies, found that, at atmospheric pressures of 20,000 feet with near zero oxygen (0.1%), there is 24-hour turtle survival in this system. He claims that there is no circulation in the turtle under these circumstances, yet it stays alive; the blood sludges, and there is no movement of blood in the blood vessels. There is also a species of ocean perch, the Arctic perch, which has no hemoglobin but gets along.

Dr. Robin: But that does not apply to the diving turtle.

Dr. Regelson: No, not to the diving turtle, but the turtle can still live 24 hours with no circulation at all before it dies.

Dr. Robin: Well, what I really tried to imply by describing the coelomic compartment and anaerobic glycolysis is that, in animals which adapt to living under conditions of oxygen depletion, one seldom finds a single mechanism to explain survival. Generally there are a number of adaptations. There is another adaptation in the turtle. When the animal dives, he decreases blood flow to his pulmonary circulation, and the blood flow then goes to what would be the equivalent of the left ventricle in man. He thereby supplies more substrate to his tissues. Apparently, to get around the problem of inefficient energy generation, one needs a number of different tricks.

Dr. Carter: I am not sure I have any quizzing. With reference to bicarbonate concentrate in the kidney, as Dr. Robin mentioned, in the final analysis the kidney must set the concentration. This has some meaning in the curves that you showed regarding acute and chronic. That is, it has been shown, for example, that the bicarbonate Tm, or the maximum amount of bicarbonate that is reabsorbed, will go up in a dog exposed chronically to increased CO_2 atmospheres. In other words, that is an adaptive mechanism. We assume, and I do not think anyone has any measurements to prove it, that the same thing occurs in man. The reason we do not have measurements that are very good is that experimentally it is very difficult to put man in a CO_2 environment. The only people that have been really successful at this are the submariners in situations where CO_2 concentration in submarines has been up around 6%. In case you have not tried it, just picking up a mask full of CO_2 is a hilarious experience, to put it mildly and that is not the least of your worries. As soon as you stop, you will feel like you are about to die. So, this is a tough human experiment.

In considering the shark, perhaps the most pertinent animal is the rat; because the rat is not like the dog. The bicarbonate Tm in the rat normally is close to 18 mM higher than the serum bicarbonate normally maintained. That is a peculiar circumstance, but it seems to be quite true. In other words, in the case of man and dog, the bicarbonate Tm is at any given moment pretty well equivalent to what the serum bicarbonate is. This is not true in the rat, where the Tm approaches the equivalent of 38 to 40 mM bicarbonate in the plasma. For this reason, when you give a rat CO_2 rebreathing, he immediately has a very rapid increase in serum bicarbonate and tends to have a somewhat blunter range of pH change, although it ultimately does become acidotic. The whole thing is over in 24 hours, whereas it may take as long as several days in the dog. I wonder whether the same thing is not true in the shark—that the Tm for bicarbonate must be at all times higher than what the bicarbonate is in the plasma. Otherwise the shark would not retain the bicarbonate when one raised the Pco_2, unless one wanted to make an inordinate curve for the action of Pco_2 in the bicarbonate Tm. Do you have any measurement of that?

Dr. Robin: The shark kidney is insensitive to any measured parameter to changes in either CO_2 tension, bicarbonate or hydrogen ion. That is to say, if one takes the animal and loads him with bicarbonate, this changes neither the urinary pH or urinary bicarbonate concentration nor urinary amonium excretion or urinary titratable acidity. The presumption is that, since his pH under these circumstances returns to normal, excretion takes place through the gill and, for reasons which are not entirely clear, the shark kidney essentially does not respond to changes in extracellular pH by "appropriate" activity. There is no possibility of defining Tm under these circumstances.

Dr. Carter: Did I understand you to say that the gill excretes bicarbonate?

Dr. Robin: Yes.

Dr. Carter: Does it do this continuously or only when bicarbonate concentration is raised? And if it does it only when the concentration is raised, at what point does one have to raise it to see bicarbonate in the gill effluent? In other words, the Tm in the gill conceivably is in excess of the serum concentration, which would amount to the same thing as the condition in the rat.

Dr. Robin: The gill does not appear to respond by an increased Tm. That is to say, the difference between the mixed venous and arterial bicarbonate concentrations multiplied by cardiac output does not increase under circumstances in which plasma bicarbonate becomes markedly raised through inhalation of CO_2. I would not want to push these data too far, because they are preliminary. It is much more simple to measure Tm in a dog kidney than in a dogfish gill, but our impression is that this is not a very sharply regulated mechanism; and I have no observations of chronic changes.

Dr. Kiesow: In regard to your data on the pond turtle, would you have, by any chance, quotients on anaerobic glycolysis in various tissues, so that one could get an idea about the possible energy production by glycolytic pathways?

Dr. Robin: The RQ for the whole animal rapidly approaches infinity. He is producing CO_2 because of buffering of hydrogen ion by HCO_3^-, and he is not using oxygen, so he is producing buffer CO_2. RQ measurements under these circumstances are not useful for the whole animal. We have some preliminary data for brain slices, and our impression is that, even under circumstances of adequate oxygen supply, the preferential route of energy generation is through anaerobic glycolysis.

Dr. Kiesow: Would you then say that turtle tissues are pretty much similar to cancer tissues?

Dr. Robin: This is a useful analogy. They act like Ehrlich acites tumor cells.

Dr. Kiesow: The fetal tissue, however, cannot survive under anaerobic conditions.

Dr. Robin: Nor can the turtle for, say, longer than two weeks. I should have made that clear. You are extending the three minutes of anoxic survival in man, to two weeks in the turtle, but he: a) runs out of buffer, and that may be one factor; and b) he may run out of substrate; and c) he may have some absolute oxygen requirement which produces structural deterioration after a longer time.

Dr. Regelson: There is a lipid chemist who did a study of cardiolipin, going on up through the animal kingdom in an effort to determine mitochondrial patterns and development of respiratory pathways in the phylogenetic sense. He came across a salamander, amphiuma, which I always wanted to work with and which has no mitochondria at all. There is deposition of a melanin pigment and crystalloid aggregates in the liver when amphiuma aestivates, the implication being that the pigment in some way has a respiratory role. Several investigators have postulated that melanin could play a role in oxidation-reduction systems to provide an alternative source of energy.

Dr. Robin: That is very interesting. I am not aware of that data.

Dr. Huf: In your earlier discussion you emphasized the need for a low Pco_2 and bicarbonate in aquatic animals because they have to maintain their oxygen requirement. I take it, then, that if one elevated the CO_2 tension, the animal would die because of interference with the oxygen metabolism.

Dr. Robin: In the limiting case, yes. If one is living on ambient oxygen so that there is an ambient PO_2 of 150, there is a Pco_2 so high that the oxygen tension must be so correspondingly low that one cannot survive.

Dr. Huf: Could one extrapolate

from here and conclude that if one infused animals with bicarbonate/CO_2 mixtures at elevated Pco_2 but not in such a manner as would interfere with their general acid-base balance, the animals could survive if one also elevated the PO_2?

Dr. Robin: This is under equilibrium circumstances, and all this relationship says is that, since nitrogen is fixed in an air-breathing animal, in essence whatever is left must be distributed between oxygen and CO_2 in the steady-state circumstance. Hence, I do not think one could extrapolate these quantitative relationships therapeutically.

Dr. Brackett: The curve you showed for chronic hypercapnia—between 40 and 60 mm Hg—showed a normal pH. Certainly it is difficult in clinical material to be sure about associated acid-base disorders. I assume that these were reasonably excluded in your patients. On the other hand, dog, as you pointed out, never reaches "normal compensation," so this may be a semantic problem, in part, but I would wonder. We, too, have been interested in studying chronic, steady-state hypercapnia in carefully selected patients. If this same linear relationship obtains and one knows, in fact, the "normal pH" in an individual, at any degree of hypercapnia he would not have the same hydrogen ion activity. I think that, certainly above a Pco_2 of 55, there might be a discernible difference. Below that it is very difficult to know when paired data are not available.

Dr. Robin: If one finds a range of pH's, and one finds pH's as high as 7.47 and 7.48, it would be hard to believe that a patient, when he was normal and his Pco_2 was 40, had a pH of 7.53 or 7.54. Secondly, as was shown originally by Dr. Schwartz, if one takes such patients and infuses bicarbonate so that one normalizes arterial pH, they promptly excrete the infused HCO_3^-. That is to say, they return to an extracellular pH which seems, for whatever regulating mechanisms there are, to be satisfactory to them. We have looked meticulously for changes in chloride balance in some of these patients to see what happens to exogenously administered chloride and looked for evidence of potassium depletion inside of muscle. While in some patients it is possible to find some kind of complicating metabolic lesion, a number of these patients with normal or elevated pH's do not show such abnormalities. The implication of the shark studies is that there is another animal that is able to generate enough bicarbonate to maintain a baseline or a normal value of pH in the face of sharp hypercapnia. Our extrapolation would be that if it can occur in some animals, then presumably it could occur in man.

Dr. Brackett: The dog data do not demonstrate complete compensation when starting with each individual dog's control pH, and in our human data, on the basis of 20 patients and 40 steady-state points carefully selected, man behaves very much like the dog, with 95% significance bonds falling about two nanomoles lower. This suggests that man reaches for but never quite attains complete compensation with Pco_2's over 55 mm Hg.

Dr. Robin. Thank you. I think this makes a very important point. In a sense, it highlights the very real differences that exist in this area. People who are very much intrigued with whole body titration curves have been intrigued with statistical analyses. It seems to me, though, that if you are interested in mechanisms, it is more important to explain the exception, the man who has the pH of 7.48 when his Pco_2 is 55, because if you fit his values in with statistics, it will just be a point buried in the standard deviation of your whole body titration curve. Yet it may mean that he has hyperventilated, that he is potassium depleted or that he has special kinds of regulatory mechanisms at work. So, in a sense, I think both approaches are important, but I like to think, although this is a subjective judgment, that one of the ways of progressing in this area is to analyze these patients in a very individual, mechanistic sense. Of course, the thing that impressed us when we did this for intracellular pH was the evidence that they regulated their intracellular pH more closely than they regulated extracellular pH.