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Preface

During the past 10 years, new and important information on cations and anions in relation to control of blood vessels has been brought forth. Although several reviews on certain aspects of this subject have appeared during the past 5 years, no single monograph or text is available for the research scientist, clinician or serious student, graduate or postgraduate, on ions and microcirculatory regulation. This information is particularly important in view of the purported role of cations and anions in vascular diseases. I have been fortunate in being able to gather so many active experts and outstanding scientists to contribute to this volume.

The contributors of this volume have attempted to: (a) critically evaluate work done in the past; (b) describe the present state of the art, and (c) point out future directions which seem profitable and challenging. It is my hope that this monograph has brought the material discussed up to date and his reviewed areas hitherto neglected.

B.M. Altura

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Role of Hydrogen Ions in Regulation of Cerebral Blood Flow and Other Regional Flows

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Principles of H^+ Homeostasis

It is a fundamental principle that the mammalian organism keeps the intra- and extracellular H^+ concentration as constant as possible. The reason for this is that normal function and metabolism of the organs is only possible in an extremely narrow range of extracellular H^+ concentrations. A small change in extracellular concentration of about 0.00005 m*M* is the extreme which may be tolerated. Compared to the extracellular changes in concentration of other ions, which can occur without major disturbance of function, the regulation of H^+ concentration must be orders of magnitude

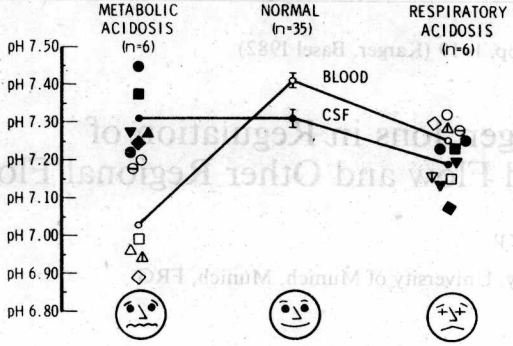

Fig. 1. Effects of metabolic and respiratory acidosis on cerebrospinal fluid pH and state of consciousness [from ref. 49 by permission].

more effective than that of other ions. The intracellular H^+ concentration is even less variable. So, we can take the changes of extracellular concentration as a sensitive indicator of what happens within the cells.

A systemic acid-base disturbance can be induced by changing either the respiratory or the non-respiratory component of the acid-base status. Whereas respiratory changes, according to their magnitude, will act on all organs, there is one organ which is not immediately and directly affected by systemic non-respiratory disturbances, i.e. the brain. This is due to the existence of a blood-brain barrier, which impedes the penetration of H^+ and HCO_3^- but not of CO_2 (for details, see later).

While the whole organism has a very effective system of keeping its H^+ concentration within narrow limits, the brain has additional mechanisms of regulation. This appears necessary, since the function of the cells of the central nervous system is critically dependent on the pH of the surrounding extracellular fluid which is contiguous to the cerebrospinal fluid. Acidosis of the cerebrospinal fluid has been shown to be deleterious to brain function in patients [49, 71]. The disturbances in brain function depended upon the degree of acidosis in the cerebrospinal fluid, not on the systemic acid-base balance. On the other hand, systemic acidosis per se did not induce any kind of encephalopathy, as long as the cerebrospinal fluid pH was not considerably decreased (fig. 1). The effects of cerebrospinal fluid acidosis on the function of the central nervous system are extremely complex [for details, see 71]. The only general statement which may be allowed is that inhalation of less than 10% CO_2 can have partly excitant

and partly depressant effects, whereas higher concentrations of CO_2 induce anesthesia [38].

Metabolic Processes Supporting H^+ Homeostasis

As a consequence of cell metabolism, there is a continuous production of acid end products. During enhanced or anaerobic metabolism, this production increases. The organism as a whole regulates the excretion of the acid end products by adjusting both the excretion of CO_2 (by ventilation) and of fixed acids (by the kidney) to the respective production to keep the total H^+ content constant. This bulk adjustment is certainly not sufficient to keep the different organs in acid-base balance when their functional activity changes more or less independently. Intracellular and extracellular buffer mechanisms can effectively reduce the changes in pH during increased or decreased production of acid metabolites. Concerning extracellular buffering, the brain is the least protected organ, since the cerebrospinal fluid surrounding the brain cells has a low buffer capacity due to its extremely low protein content. In spite of this fact, the pH of the cerebrospinal fluid seems to be subjected to less fluctuation compared to the extracellular fluid of organs other than the brain. This is mainly due to the strong pH sensitivity of the cerebral vessels and to the effective regulation of the HCO_3^- concentration in the cerebrospinal fluid. In addition to these mechanisms, which are discussed in more detail later, another pH homeostatic principle seems to be working generally equally in different organs, that is a pH-sensitive regulation of metabolism [13, 14]. According to this mechanism, the CO_2 and lactate production can, by negative feedback, influence the carbohydrate metabolism of the tissue. An acidic shift of the pH in the cell decreases lactate and pyruvate production, thus reducing H^+ generation. H^+-consuming processes, like oxidation of glutamine and glutamate, are increased. The contrary happens with an alkaline pH. As a consequence of this mechanism, each process which generates or consumes H^+ contributes to its own negative feedback control. The mechanisms by which this feedback control might work have been investigated in rat brains in vivo during exposure to high CO_2 [19, 20, 44]. Without going into details, a primary effect of increased CO_2 tensions seems to be inhibition of the glycolytic rate at the phosphofructokinase step. Such an effect of acidosis holds also for skeletal muscle [63], erythrocytes, leukocytes and tumor cells [for references see 70]. Conversely,

alkalosis stimulates glycolysis by enhancing the phosphofructokinase step [55, 63, 64, 70]. The inhibition of phosphofructokinase by acidosis leads to a decreased delivery of pyruvate and lactate, thus reducing a H^+-generating step. Besides the carbohydrate substrate depletion, the amino acid pool is reduced, which is a process involving H^+ consumption. Examples for H^+-generating and H^+-consuming metabolic processes:

Neutral
$$\text{Glucose} + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

H^+ Generating
$$\text{Glucose} \rightarrow 2\ \text{Lactate}^- + 2\ H^+$$

H^+ Consuming
$$\text{Lactate}^- + 3\ O_2 + H^+ \rightarrow 3\ CO_2 + 3\ H_2O$$
$$\text{Glutamate}^- + 4{,}5\ O_2 + 2\ H^+ \rightarrow 5\ CO_2 + 3\ H_2O + NH_4^+$$
$$2\ \text{Lactate}^- + 2\ H^+ \rightarrow \text{Glucose}$$
$$2\ \text{Glutamate}^- + 3\ O_2 + 4\ H^+ \rightarrow \text{Glucose} + 4\ CO_2 + 2\ NH_4^+$$

Effect of CO_2 on Cerebral Vessels and Cerebral Blood Flow

One of the easiest experiments in studies of cerebral blood flow and cerebrovascular reactions is to test the effect of changing the arterial CO_2 tension. Consequently, this type of experiment has been performed by several generations of investigators. The results of these experiments showed a direct correlation between arterial CO_2 tension and cerebral blood flow or pial arterial diameter. These results are easily reproducible not only during the awake state, but also on a lower level of sensitivity during deep barbiturate anesthesia [21] and hemorrhagic hypotension [30]. Extensive references can be obtained from the books of *Wyke* [71] and *Purves* [50]. Quantification of the CO_2 effect for awake man is given by *Olesen* et al. [47] and amounts to 4% change in cerebral blood flow with 1 mm Hg change in arterial CO_2 tension. Physiologically, large alterations in systemic CO_2 tension may occur only as an exception, since central and peripheral chemoreceptors yield an effective regulation of this parameter. However, these experiments can mimic the effects of CO_2 not when given by inhalation but when accumulating as a consequence of brain metabolism. The end product of brain cell metabolism can easily diffuse over the brain cell membrane and gain access to other brain cells, to their surrounding cerebrospinal fluid, to the vascular walls of brain vessels, and to the blood perfusing the brain (fig. 2). Thus, each of these structures could contain receptors yielding information to the effectors which are the vas-

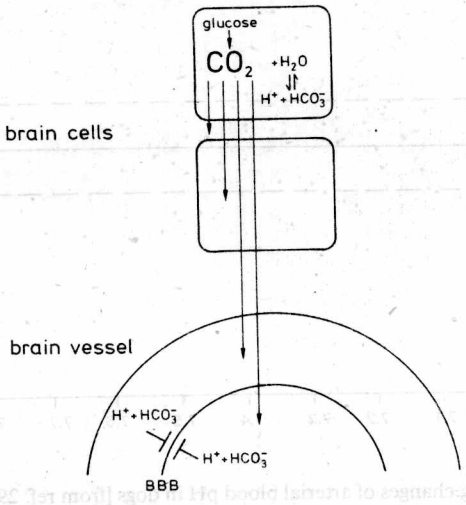

Fig. 2. Schematic representation of the formation and distribution of CO_2 in the brain tissue. BBB = blood-brain barrier.

cular smooth muscle cells in the cerebral arteries and arterioles. Experiments in which the arterial CO_2 tension was changed within seconds showed changes in calculated cerebral blood flow which occurred at the earliest time at which CO_2 could be expected to have reached the brain arteries and arterioles [58]. Although there exists some criticism on the applied calculations of cerebral blood flow from the arteriovenous difference for O_2 during changes in CO_2 tension [35], these experiments can be taken as indication that tissue CO_2 tension and venous CO_2 tension are not the triggers for the reaction of cerebral resistance vessels to CO_2, and that the receptors must be located close to or in the arterial or arteriolar wall of brain vessels. A more exact description of the site and mechanism of action of CO_2 will be possible when the effects of pH on the cerebrovascular resistance have been described.

The pH Hypothesis for the Control of Cerebrovascular Resistance

The experiments employing changes in arterial CO_2 tension left open the question of whether CO_2 acts directly (in molecular form) on the cerebral vascular smooth muscle cells or by changing the pH either in the fluid outside or inside the vessels. Experiments investigating the question

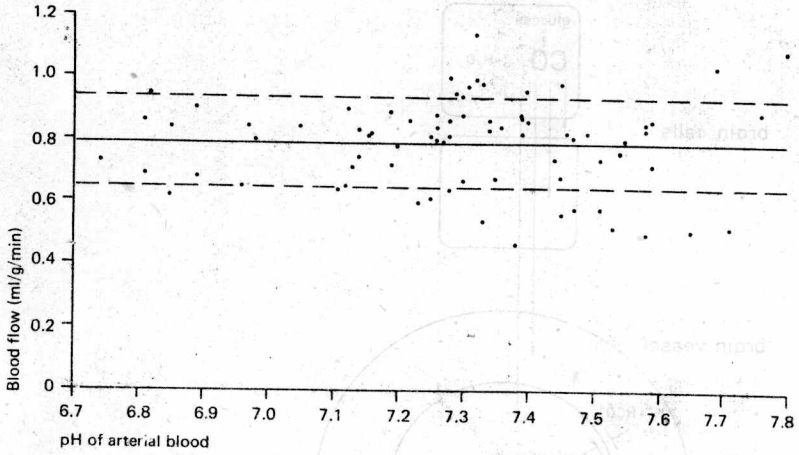

Fig. 3. Cortical blood flow during changes of arterial blood pH in dogs [from ref. 29 by permission].

whether a change in blood pH could also induce changes in cerebral blood flow have demonstrated that arterial pH can be varied over a wide range without inducing changes in cortical blood flow as long as the arterial pCO_2 is not changed (fig. 3) [29]. The explanation is that CO_2 diffuses rapidly through the blood-brain barrier to gain access to the cerebrovascular smooth muscle cells, whereas an acute metabolic acidosis or alkalosis of blood primarily changes the blood concentrations of H^+ and HCO_3^-, which are both not easily permeable at the blood-brain barrier (fig. 2).

From the experiments employing changes in the respiratory or metabolic component of the acid-base status in the blood, it can be concluded that the cerebrovascular resistance can be influenced only by CO_2, or by the brain extracellular fluid pH, or by both. The following experimental evidence led to the formulation of the pH hypothesis: (1) an inverse correlation could be found between brain cortical extracellular pH and blood flow during acute changes in blood acid-base status [6] and between mock spinal fluid pH and pial vessel reactions [17]; (2) a high CO_2 concentration given to the exposed cortex induced an increase in cortical blood flow [24]; (3) cerebrospinal pH and cerebral blood flow also correlated well during prolonged changes in acid-base balance [1, 18, 57, 61]. This unifying hypothesis, which was formulated at that time by several authors, e.g. *Lassen* [42] and *Skinhoj* [60], claimed that the cerebrospinal fluid pH is

the main factor controlling cerebral blood flow. The pH hypothesis has the advantage that it offers a simple explanation for the adjustment of cerebral blood flow to the metabolic demands: an enhanced metabolism would lead to an increase in CO_2 and/or lactate concentration, and this would induce a dilation of cerebral resistance vessels. The hypothesis of a metabolic feedback control of cerebrovascular resistance first formulated by *Roy and Sherrington* [53] nearly 100 years ago principally corresponded to the adenosine hypothesis formulated by *Berne* [4] for the coronary circulation.

The experimental evidence on which the pH hypothesis was based was rather scanty. It was apparent that the experimental finding of a relationship between cerebrospinal fluid H^+ concentration and cerebral blood flow under some, not all, experimental conditions did not allow any statement on the causality of this relationship. For testing the validity of the pH hypothesis two major types of experiments have been performed. One type was done to make sure that changes in extravascular pH can indeed influence the diameter of cerebral arteries. The other type was performed to see under which conditions a change in brain extracellular fluid H^+ activity can be measured. Whereas the experimental evidence concerning the first type is convincing, there are still controversies about the experiments of the second type, which are discussed in the following chapters.

Evidence for a Direct Action of H^+ on Cerebral Resistance Vessels

The above-cited experiments of *Elliott and Jasper* [17], who flushed the cerebral surface with solutions of varying pH and observed corresponding changes in pial vessel caliber, gave a strong indication of a vasoactive effect of H^+. Since this method does not exclude secondary vascular effects exerted by possible actions of the solutions on the metabolism of the underlying brain tissue, *Ingrar* and *Lassen* have introduced a refinement of the method. The mock spinal fluids were applied under microscopic control to the outside of single pial arteries using micropuncture technique established in kidney physiology [66]. These experiments confirmed the results of *Elliott and Jasper* [17]. Since the pH of the solutions tested was varied over an extreme and unphysiological range, it was shown in a subsequent paper [41] that more physiological changes in perivascular HCO_3^- concentration could also induce pial arterial reactions. These results have been confirmed by several groups [5, 25, 36]. Simultaneous microapplication of mock cerebrospinal fluids with varying

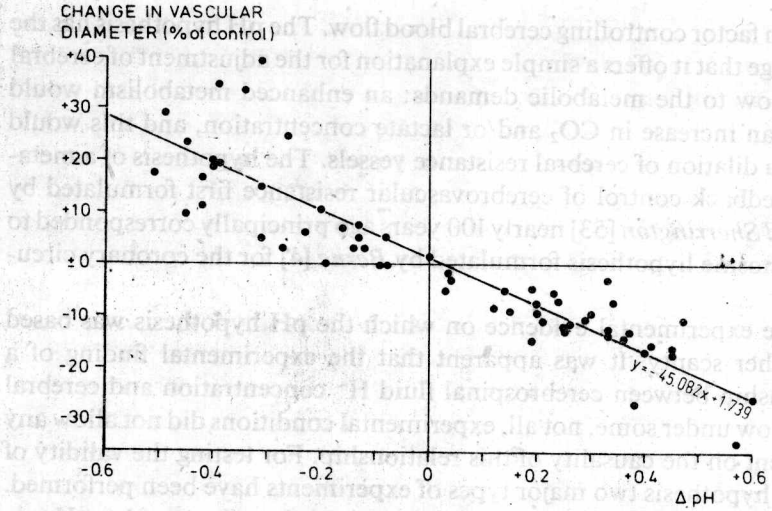

Fig. 4. Correlation between changes in the perivascular pH of single pial arteries of cats with the changes of their diameter [from ref. 56 by permission].

HCO_3^- concentrations to single pial arteries and measurement of both perivascular H^+ activity at the injection site, using pH sensitive microelectrodes, and of pial arterial diameter demonstrated that the perivascular pH was indeed changed by altering the HCO_3^- concentration of the mock cerebrospinal fluids (fig. 4) [56]. That it is indeed the change in pH which is the trigger for pial arterial reactions could be convincingly demonstrated by *Kontos* et al. [36]. They showed that manipulations of the HCO_3^- concentration and the CO_2 tension of the mock cerebrospinal fluid only induced vascular reactions of pial arteries when the pH of the solutions was altered. Hydrogen ion-dependent reactions exist also at resistance vessels which are located inside the brain, although secondary effects cannot be excluded in these experiments. When the ventricular system was perfused with solutions of varying HCO_3^- concentrations, changes in blood flow could be detected in the caudate nucleus [48]. Intraparenchymal injections of mock cerebrospinal fluids into the hypothalamus, combined with local measurements of hypothalamic blood flow, yielded similar results [8]. All data, when taken together, and the fact that vascular reactions start within seconds after changing the pH in the perivascular fluid [25, 41], are strong

evidence that the resistance of cerebral arteries and arterioles can be regulated very effectively and quickly by changes in their perivascular H^+ activity.

The Question of Additional, Indirect Mechanisms at Cerebral Vessels

The described experiments have unequivocally demonstrated a direct vasoactive action of H^+ at cerebral resistance vessels. The high degree of sensitivity and the quick responsiveness of cerebral arteries and arterioles appear sufficient for a physiological regulation exerted by direct effects of extravascular pH on pial arterial smooth muscle cells and do not necessitate additional indirect mechanisms to support or enlarge these direct effects. However, a large number of papers has been published during the last years investigating the question of such additional mechanisms. The varying and contradictive results have been summarized in several recent review articles [16, 39, 51]. The respective experiments were based on the assumption of a reflex pathway mediating the reaction to the pH (mostly CO_2) stimulus. Such a pathway would include chemoreceptors and afferent nerve fibers as well as a brain stem center and efferent nerve fibers contacting cerebral vessels.

As far as the question of a pH effect on cerebral vessels is concerned, these experiments have not given any suggestion which was not contradicted by other experiments. This could be due to the fact that many of these experiments need major surgical interventions which can compromise the normal regulation of cerebral blood flow leaving the possibility of loss of physiological vascular reactions as well as of artifactual reactions. In my opinion, a very simple experiment performed by *Kontos* et al. [36] makes any significant role of reflex pathways in this context unlikely. These authors induced vasodilation in pial arteries by increasing the arterial CO_2 tension (CO_2 breathing). When the space under the pial window was flushed with a mock cerebrospinal fluid in which the pCO_2 was reduced from normal by an amount equal to the rise in arterial blood pCO_2, the vasodilation was completely abolished. The arteries, which were exposed to high CO_2 tensions from the blood side and corresponding low CO_2 tensions from the cerebrospinal fluid side, showed exactly the same diameters as under control conditions with normal CO_2 tensions. Since the changes of mock cerebrospinal fluid pH were completely local and confined to the space under the pial window, reflex mechanisms could be expected to be still activated and should have prevented pial arterial

diameter from returning to its control value. The conclusion seems justified that the action of CO_2 and pH on pial vessels is completely local.

Brain Extracellular pH and Tissue Lactate during Conditions of High Cerebral Blood Flow

As already outlined, a verification of the pH hypothesis requires, besides the demonstration of the vasoactivity of extravascular H^+, changes in the concentration or activity of H^+ to be measured in brain extracellular fluid or tissue under conditions in which cerebral blood flow is changed physiologically. Such conditions are mainly changes in neuronal activity, which should be accompanied by corresponding changes in H^+ activity in the surrounding cerebrospinal fluid if H^+ mediate the coupling between metabolism and blood flow. An acidosis of cortical tissue or cerebrospinal fluid has, indeed, been measured by most of the investigators for longer-lasting phases of extreme neuronal activation (seizure activity) [for references see 10, 34]. However, to accept H^+ as coupling factors would necessitate their release from tissue simultaneously with the increase in cerebral blood flow, which would mean a release of H^+ within the first seconds of activation. This is just the point where the conclusions of different groups are divergent. Although increases in tissue lactate concentration have been measured within seconds after physiological [26, 54] and electrical or pharmacological stimulation [7, 11, 12, 15, 34, 46], these increases may not induce tissue acidosis if the pCO_2 is decreased simultaneously, as has been suggested [11, 46]. A better answer than given from calculations might be expected from direct measurements of H^+ activity in the cortical tissue. However, the results of the studies using pH electrodes need some consideration. When the electrodes are placed on the brain surface [10, 34] the arachnoid membrane serves as a diffusion barrier which makes a quick release of H^+ from brain tissue unmeasurable by the electrode [34]. On the other hand, insertion of the electrodes into the brain [3, 33, 65] creates some space of dead tissue around the electrode [3] which could delay the diffusion of H^+ from functioning tissue to the electrode. Thus, the results obtained may not be conclusive for the first seconds of cortical activation, but they show congruently cerebral tissue acidosis starting between 0 and 20 s after cortical activation. When the increase in blood pressure, which normally occurs during pharmacological (bicuculline) induction of seizure, is avoided, an immediate acidosis with no delay can be measured in the cortical subarachnoid space when the microelectrodes only penetrate the superficial arachnoid layer (fig. 5) [40].