THE REGULATION OF RESPIRATION

IV. TISSUE ACIDITY, BLOOD ACIDITY AND PULMONARY VENTILATION. A STUDY OF THE EFFECTS OF SEMIPERMEABILITY OF MEMBRANES AND THE BUFFERING ACTION OF TISSUES WITH THE CONTINUOUS METHOD OF RECORDING CHANGES IN ACIDITY

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Received for publication June 28, 1926

Several years ago experiments by one of us pointed to a lack of correspondence between blood acidity and pulmonary ventilation, which seemed to contradict the generally accepted view that the acidity of the arterial blood controlled respiration. These experiments (1) led to the statement that "The conception that the hydrogen ion concentration of the arterial blood regulates respiration is analyzed and found wanting;" that "Parallelism between pulmonary ventilation and arterial pH is an accidental occurrence prevailing only under special conditions."

Considering respiratory control as a problem in transportation—the blood carrying acid to and from the cells and the metabolism of the cells determining the kind and amount of acid leaving and entering the cells—there was no reason for assuming a constant relation between blood and tissue acidity. It was pointed out that the more acid arterial blood may be the more efficient in carrying acid away from the tissues, that blood acidity can at best be only an indirect criterion of tissue acidity, that blood acidity and tissue acidity may vary in opposite directions as well as in the same direction. An effort to correlate the net effect of innumerable factors involved in changes of blood and tissue acidity under a variety of conditions pointed to a high coincidence of a direct relation of pulmonary ventilation to the acidity of the respiratory center itself. This led to the advancement of a working hypothesis connecting tissue acidity rather than blood acidity with the behavior of the respiratory center.

As to the lack of correspondence between pulmonary ventilation and blood acidity—that must be accepted as a fact. The graphic record of changes in acidity of the circulating blood leaves no room for doubt (2)

1This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.
TISSUE ACIDITY, BLOOD ACIDITY AND PULMONARY VENTILATION

Results with the manganese dioxide electrode in hemorrhage and subsequent injection, mechanical asphyxia during the administration of room air and pure oxygen, the administration of sodium bicarbonate, ammonium chloride, adrenalin, nitrogen, etc., show the lack of correspondence between blood acidity and pulmonary ventilation. An inverse relation between blood acidity and pulmonary ventilation is by far the more common.

On the other hand a causal relation between tissue acidity and pulmonary ventilation should be considered as theory. And to avoid a common mistake of accepting theory as fact it is well to recall that the problem of relating functional activity of nervous structure such as the respiratory mechanism to fundamental processes still remains to be done (3).

One need only consider the effects of anesthetics and various salts on the respiratory center to realize that more than one factor plays a part in the control of respiration. We say that anesthetics depress the excitability of the respiratory center. During anesthesia there is a lack of oxygen, excess of carbon dioxide and hydrogen ions; nevertheless, respiration is lower than normal. But on the other hand, aside from the depression of activity, the center seems to follow the same general laws of response to respiratory stimuli.

The limited space prevents a discussion of fundamental problems involved in the automatic and reflex activity of nerve tissue. It should be recalled that the regulation of respiration in its ultimate analysis may be found to be an electrical phenomenon occurring with the aid of a surface membrane and change in composition of the fluids on both sides of that membrane. Whether acid is the only reagent which can produce the necessary electrical disturbance involved in the nerve impulse is highly improbable; on the other hand, it may be the agent most employed in the body.

The purpose of this research is to determine how closely theory is related to fact with respect to the effects of carbon dioxide, sodium carbonate and sodium bicarbonate. It represents an effort to learn, with various methods of approach, the directional changes in tissue acidity with synchronous changes in blood acidity; to follow the effects of permeability of cell membranes on tissue acidity and to ascertain the buffering action of tissues.

METHOD. The experiments were performed on dogs anesthetized with hypodermic injection of 10 mgm. morphine sulphate per kgm. body weight followed by rectal injection of 0.8 gram urethane per kgm. body weight. These injections usually maintain the animal in good anesthesia without marked depression of either respiration or circulation. Occasionally subsequent injections of urethane are necessary to insure greater regularity of breathing. Synchronous records of the acidity of the circulating arterial and venous blood were made with the manganese dioxide electrode as described in the preceding paper (4). The electrode vessels were placed in the course of the carotid artery and external jugular vein. In some experiments changes in acidity of the cerebro-spinal fluid were also recorded with the electrode vessel shown in figure 1. The original vessel consists of
a small glass T-tube, the vertical tube serving to hold the manganese dioxide electrode, and the right angle tube, filled with saline solution, serving to close the potentiometer circuit. The dog is placed on its side and the occipito-allantoid membrane and dura over the fourth ventricle are exposed by reflecting the neck muscles with thermocautery. A pursestring suture is made over the center of the membrane and the lower end of the electrode vessel, which is provided with a ring of DeKhotinsky cement, is passed through a small incision into the fourth ventricle. The pursestring suture is then secured and about 50 grams traction applied to the electrode vessel with string and pulley at right angles to the membrane. This prevents injury and hemorrhage by keeping the point of the electrode and the electrode vessel away from the floor of the fourth ventricle despite gross movements of the head of the animal during heavy ventilation. The greatest precaution should be employed to prevent hemorrhage as the presence of blood, due to the effects of oxidation and reduction, influences the change in acidity of the cerebrospinal fluid. For purposes of checking the validity of the data provided by the manganese dioxide electrode an additional tube was cemented with DeKhotinsky cement to the electrode vessel to provide samples of cerebrospinal fluid. The pH of the samples was determined with the quinhydrone electrode, as in the preceding paper. Blood pressure was recorded with the mercury manometer and pulmonary ventilation with rebreathing tanks. The animal was connected by a single inspiratory and expiratory valve with three tanks of seventy liters capacity each. This arrangement allows a quick shift from one gaseous mixture to another. The tanks are provided with soda lime cartridges so that basal metabolism as well as pulmonary ventilation may be followed. The rate of oxygen consumption is indicated by the gradient of the respiratory record.

RESULTS. Figure 2 shows the effects of administration of carbon dioxide directly into the trachea while the animal was connected with a rebreathing arrangement. The cerebro-spinal fluid electrode and sampling vessel has been markedly improved by constructing it from a block of transparent bakelite. The ring of deKhotinsky cement which has pulled off the glass vessel during the course of several experiments is replaced by a 30° angle-shaped groove. On tightening the purse string suture the membrane fits snugly in the groove. The sampling tube is of finer bore just permitting the passage of the syringe needle. The upper end of the sampling tube opens on an offset which is covered with rubber dam.
tank filled with room air. The upward bend of the respiratory record is the result of the administration of carbon dioxide and the sudden downward bend is due to the absorption of the gas at the end of administration. In all subsequent records gaseous mixtures are administered as described above.

The effects of administration of carbon dioxide shown in figure 2 are increased acidity of the arterial and venous blood accompanied by increased pulmonary ventilation. These effects are followed during recovery by decreased acidity of the arterial and venous blood and decreased pulmonary ventilation. The sequence of events is designated. The electrode in the carotid blood indicates increased acidity first and is followed in about ten seconds by the same indications in the jugular blood. Careful measurement of the respiratory record shows that the arterial electrode responds to increased carbon dioxide before the respiratory center, and, similarly, that the venous electrode responds to increased carbon dioxide after the respiratory center. That is, the respiratory center which is midway between the two electrodes responds when the carbonated blood arrives at the center midway between points 1 and 2 on the figure.

Figure 2 represents the type of data which was largely responsible for the view that acidity of the blood controls pulmonary ventilation. The stimulating effects of the carbon dioxide and the depressing effects of sodium carbonate represent two of the few instances of parallelism between arterial acidity and pulmonary ventilation, and it is shown that in these two instances the changes in blood acidity reflect similar changes in tissue acidity. Accepting the view that tissue acidity (in this case acidity of the respiratory center) is the dominant chemical factor controlling respiration, an explanation of the effects of carbon dioxide becomes apparent (fig. 2). In following the shape and magnitude of the arterial and venous acidity curves one is impressed with the greater change and the greater abruptness of the change in acidity in the arterial blood both during the administration of carbon dioxide and during recovery. It is possible that the greater magnitude of the acidity change on the arterial side may be explained in part by the effects of reduction of oxyhemoglobin on the passage of the blood from the arterial to the venous side of the tissues but the change in the gradients of the acidity curve resulting from the flow of blood through the tissues seems explainable primarily by the passage of carbon dioxide between the blood and tissue. The record indicates that it requires an appreciable amount of time for the tissues to saturate with carbon dioxide on administration and to desaturate during recovery. (Note the very late recovery on the venous side.) In that event the venous blood must contain less carbon dioxide than the arterial blood during the administration of carbon dioxide and more during recovery; that is, the tissues have acted as buffers to the blood and in the process have temporarily become more acid themselves.
An exaggerated example of similar conditions is shown in figure 3, illustrating the effects of a prolonged administration of a twenty per cent mixture of carbon dioxide in room air. The arterial change in acidity is again abrupt, and recalling the appreciable time required for the manganese dioxide electrode to come into equilibrium with the blood itself, it is obvious that the arterial blood comes into equilibrium with the change in alveolar gases more completely than the record suggests. On the whole all our records indicate that equilibrium is rapidly established between the blood and the alveolar gases and only slowly between the blood and the tissues,
indicating the great capacity of the tissues to buffer. In figure 3 the venous record again shows a relatively gentle gradient on the administration of carbon dioxide, indicating a progressive saturation of the tissues with the gas. This in turn is followed by a gentle recovery gradient which indicates a progressive desaturation of the tissues. Recovery of the venous blood to the original acid level is decidedly incomplete within the limits of the published record.

Not infrequently the acidity of the arterial blood may fall below the normal level during recovery. An explanation of this and other details will be left for later papers.

Figures 4, 6, 8 and 11 show the effects of intravenous injection of sodium carbonate. The effects are increased alkalinity of the arterial and venous blood, decreased respiration and a fall in blood pressure followed by decreased alkalinity of the arterial and venous blood, increased respiration and increased blood pressure. Making the same comparison as in the records showing the effects of carbon dioxide it is noted that the arterial changes precede the respiratory response which in turn precedes the venous change. The arterial changes in pH are likewise greater and more abrupt than the changes in the venous blood. The records indicate that the tissues have acted as buffers to the alkalinized blood as well as to the acidified blood. Another such example is found in figure 5 which shows the effects of the intravenous injection of sodium hydroxide.

In contrast to the experiments on the administration of carbon dioxide the smaller change in pH in the venous blood cannot be attributed to the alkaline effect of the reduction of oxyhemoglobin, for in this instance the venous blood turns less alkaline than the arterial. The smaller increase in alkalinity of the venous blood may, therefore, be attributed in the main to
the buffering action of the tissues. Conceivably this buffering may occur as a result of the passage of base from the blood into the tissues and of the passage of carbon dioxide from the tissues into the blood. Either process would turn the blood more acid and the tissues more alkaline and according to theory account for the changes in pulmonary ventilation and blood pressure. The relative contribution of each of these two processes to the changes in tissue acidity and the behavior of respiration and circulation is perhaps suggested in a qualitative way by some of the later experiments to be described in this paper.

The next few records are only a further indication of the extent and significance of the buffering action of the tissues. Varying degrees of buffering by the tissues can be demonstrated by varying the diffusion gradient of the radicles involved in acid-base equilibrium between the blood and tissue. A rapid injection of sodium carbonate will momentarily lower the carbon dioxide tension of the arterial blood more than a slow injection, and, similarly, it will raise the concentration of base in a circulating block of blood more than a slow injection. In figure 4 the buffering effect of the tissues with a rapid injection is enormously greater than with a slower injection. The greater fall in blood pressure and diminution of respiration with the rapid injection, according to theory, would be explained by a greater increase in tissue alkalinity.

The buffering action of the tissues may be varied in a somewhat similar way as illustrated in figure 6. Reasoning that a slower flow of blood should permit the establishment of a more perfect equilibrium between blood and tissue on the injection of sodium carbonate and thereby increase the buffering action of the tissues, equal amounts of sodium carbonate were injected at equal rates before and after clamping both carotid arteries. The upper record shows the arterial and venous changes in alkalinity resulting from an injection with the normal volume flow of blood. The lower record shows the pH changes in the venous blood produced by injection when both carotids were clamped. (As the arterial electrode is in the carotid artery the arterial record is missing.) In the lower record the pH change in the venous blood comes decidedly later and is appreciably smaller than in the upper record. The later appearance indicates the slowing of the blood stream and the smaller change indicates the greater buffering by the tissues.

The effect of carbon dioxide on arterial and venous pH shown in figure 7 before and after hemorrhage is another demonstration of the variability of the buffering action of the tissues dependent upon the volume-flow of blood. In such experiments the effects of volume-flow of blood on changes in acidity of the arterial blood within the lungs are shown as well. Note the steepness of the arterial curve during administration of carbon dioxide in the lower record following hemorrhage as compared with the upper

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record preceding hemorrhage. It seems only reasonable to expect a more complete saturation of the blood with carbon dioxide if greater opportunity

Fig. 5. Intravenous injection of 13.5 cc. 0.4 M NaOH. Weight of dog, 8.5 kgm. 
V., jugular blood acidity curve; A, carotid blood acidity curve; P.V., pulmonary ventilation; T, time in six second intervals. Total time, 10 minutes.

Fig. 6. Effect of volume-flow of blood on the buffering of the blood by the tissues. Records A and C show the changes in alkalinity of the carotid and jugular blood on the intravenous injection of sodium carbonate with both carotids open. Record B is a corresponding curve showing the effect of occlusion of both carotids on the alkalinity change in the venous blood. A, carotid blood acidity curve; V, jugular blood acidity curve. The arrows indicate the moment of injection.

Fig. 7. Effect of volume-flow of blood on the buffering action of the tissues on the administration of carbon dioxide. The upper record shows the effects of administration of carbon dioxide before hemorrhage and the lower record after hemorrhage. P.V., pulmonary ventilation; CO₂—duration of administration of carbon dioxide; A, carotid blood acidity curve; B.P., mean blood pressure; V., jugular blood acidity curve.

Fig. 8. Changes in acidity in the tissues produced by the administration of carbon dioxide and sodium carbonate. In records A and B the manganese dioxide electrode was placed between the fibers of the sartorius muscle. In record C the electrode was placed between the cerebral convolutions.
is offered for absorption. And, similarly, it seems as reasonable that the tissues will have greater opportunity to absorb this carbon dioxide when the blood flows more slowly through the tissues. Hemorrhage then should sharpen the arterial curve and blunt the venous curve (see fig. 7).

Granting that the tissues are functioning in this buffer capacity the effects of changes within the blood stream exerted outside the blood stream should be demonstrable. Figure 8 shows changes in acidity recorded with the manganese dioxide electrode placed between the fibers of the sartorius muscle on the administration of carbon dioxide (record A) and on the injection of sodium carbonate (record B). The changes in pH are smaller and occur more slowly than the changes in the blood. In record C, however, where the electrode was placed between the cerebral convolutions the change is large and rapid.

The freedom of movement of carbon dioxide through cell membranes and its rapid rate of diffusion in solution suggests the simplest explanation of buffering action of the tissues, but a consideration of the movement of base and the restraints offered to that movement is equally essential. Whether the restraint offered to the movement of base is of the nature of a slow diffusion or of the nature of a barrier in the form of impermeability of membranes, the local effect is momentarily the same. The stratification of two solutions of the same pH $\text{H}_2\text{CO}_3/\text{NaHCO}_3 = 1/20$ and $\text{H}_2\text{CO}_3/\text{NaHCO}_3 = 3/60$, results in a change

Fig. 9. Comparative buffering effects of the tissues on the blood following the administration of sodium carbonate, sodium bicarbonate, and carbon dioxide. Greater similarity in the arterial and venous acidity curves are frequently obtainable on the injection of sodium bicarbonate. B.P., blood pressure; V, jugular blood acidity curve; A, carotid blood acidity curve; CO$_2$, duration of administration of carbon dioxide.
of pH at the interface comparable to the change which would occur if the solution were separated by a semipermeable membrane (5). The significance of restraints to movement of base, whatever the nature of the restraint may be, is indicated in figure 9—a continuous record showing the effects of injection of sodium carbonate, sodium bicarbonate and carbon dioxide. The typical buffering effects of the tissues on the injection of sodium carbonate and on the administration of carbon dioxide are present. But on the injection of sodium bicarbonate the buffering effects of the tissues are absent. There is a great similarity in magnitude and contour of the arterial and venous acidity curves which agree with the results anticipated. It will be recalled that one of us, on a previous occasion, suggested that the so-called "specific" action of sodium bicarbonate and carbon dioxide (Collip (6), Dale and Evans (7) and others) might be explained on an acid basis.

The argument presented at that time is so essential for an understanding of the present paper that it is given in part again (1).

We have recently (5) demonstrated a striking disturbance in the hydrogen ion equilibrium of a solution without the use of a membrane. We prepared a relatively weak and a relatively strong solution of sodium bicarbonate and with the aid of phenol red and carbon dioxide raised the hydrogen ion concentration of each to the same point. The two solutions were carefully stratified in a cylindrical separatory funnel. The denser solution being below, none of the constituents of the solutions can mix except by the process of diffusion. In a few moments a light colored acid ring appears at the interface. This ring gradually increases in width and simultaneously a dark alkaline ring develops below. The formation of colors widens and slowly ascends.

The explanation of the phenomena is, of course, obvious. A substance in solution tends to diffuse from a point of high concentration to a point of lower concentration. Sodium bicarbonate and carbon dioxide will, therefore, tend to diffuse upwards, but as a result of the higher rate of diffusion of carbon dioxide the disproportionate entrance of carbon dioxide in the upper solution increases the \[ \frac{\text{H}_2\text{CO}_3}{\text{NaHCO}_3} \] ratio, and, similarly, the disproportionate departure of carbon dioxide from the lower solution decreases the ratio with a consequent display of colors. An additional differential barrier, for example, the impermeability of the membrane to the sodium bicarbonate, should of course accentuate the exceedingly rapid process exemplified by the simple diffusion experiment. Undoubtedly, such hindrance occurs between the blood and the nerve cell.

Another possible explanation also occurred to us. It is known that when carbon dioxide is dissolved in water it is present in three different forms—dissolved \( \text{CO}_2 \), undissociated \( \text{H}_2\text{CO}_3 \) and dissociated \( \text{H}_3\text{CO}_3 \). Thus \( \text{CO}_2 \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}_3\text{CO}_3 \). Addition of sodium bicarbonate pushes the reaction to the left increasing both the dissolved \( \text{CO}_2 \) and the undissociated \( \text{H}_2\text{CO}_3 \). This effect alone, neglecting the formation of carbon dioxide in the tissues, would tend to produce diffusion of carbon dioxide into the tissues; that is, the injection of sodium bicarbonate should theoretically produce acidosis along with alkalemia by virtue of the greater accumulation of carbon dioxide in the tissues.

But this conception neglects entirely the effects of the \( \text{H}^- \) and \( \text{HCO}_3^- \) ions. We
know, for example, that the H ion, within the blood at least, shifts with extreme rapidity between the cells and the plasma with every circuit of the blood. If so, there appears to be no reason why dissociated carbonic acid should not also diffuse which suggests another possible stimulating effect of sodium bicarbonate.

If we stratify a solution of hydrochloric acid with water, a definite number of chlorine ions will tend to leave the acid for the water. In so doing they will pull the hydrogen ions with them and turn the water acid. This process is, of course, accelerated by the further addition of chlorine ions in the form of hydrochloric acid to the acid solution. The addition of neutral sodium chloride is also effective in accelerating the diffusion of the acid. This addition increases the number of chlorine ions tending to leave the acid solution for the water, but inasmuch as the chlorine ions cannot leave by themselves, they will take either H or Na ions with them. The H ion being the more mobile is the more easily pulled from solution and in consequence acid diffuses more rapidly than before.

It is obvious that the conditions are somewhat different in the case of a weak acid, such as carbonic acid, and its salt, sodium bicarbonate, for the addition of the salt markedly reduces the hydrogen ion concentration of the solution. Yet in so doing it has not only increased the amount of freely diffusible undissociated CO$_2$ and H$_2$CO$_3$ but has increased the rate of diffusion of the H ions which have been decreased in number. So far as the acidity of the interior of the living cell placed in such a solution is concerned, the effectiveness of the remaining hydrogen ion is theoretically increased for presumably the relative impermeability of the cell membrane to the sodium ion will prevent an accelerated diffusion of the added bicarbonate. Theoretically, at least, the intravenous injection of sodium bicarbonate should exert a double effect, preventing the diffusion of carbon dioxide from the tissues into the blood, by increasing the concentration of H$_2$CO$_3$ and CO$_2$ in the blood and preventing the diffusion outwards from the cells of the HCO$_3^-$ ion to which the H ion is attached.

Returning to the significance of figure 9 of this paper—if base is hampered in its movement from blood to tissue, the tissues would buffer mainly through a movement of carbon dioxide (consideration of the shift of chlorine and other anions is omitted). But, on the other hand, if the movement of carbon dioxide from tissue to blood is also hampered buffering is prevented on this score too. In that event the blood may pass through the tissues with approximately the same acidity curve with which it entered the tissues, and such is the common finding in a large number of experiments.

By placing an electrode in the fourth ventricle and recording the changes in acidity of the cerebro-spinal fluid perhaps a better opportunity is offered for studying the restraining effects on the movement of base between blood and tissue. In such experiments a living membrane is interposed between the blood and the electrode in the tissue fluid. Such experiments have value in that they may indicate the directional changes in tissue acidity associated with changes in blood acidity.³

³ An occasional difficulty to the continuous method of recording changes in acidity of the cerebro-spinal fluid is the rapid E.M.F. drift and dissolution of the electrode. The manganese dioxide plating may completely dissolve within an hour though
Figure 10 illustrates the effects of administration of carbon dioxide: increased acidity of the arterial and venous blood and cerebro-spinal fluid,

Fig. 10. Effects of administration of carbon dioxide on the acidity of the arterial and venous blood and the cerebro-spinal fluid. There is a very extensive drift in E.M.F. in the cerebro-spinal fluid electrode which must be corrected for as described in the preceding paper. B.P., mean blood pressure; A., carotid blood acidity curve; C.S.F., cerebro-spinal fluid acidity curve; V., jugular blood acidity curve; P.V., pulmonary ventilation; CO₂, duration of administration of carbon dioxide.

Fig. 11. Effects of intravenous injection of sodium carbonate on the acidity of the arterial and venous blood and cerebro-spinal fluid. Note the drift in the cerebro-spinal fluid acidity record. Weight of animal 10.5 kgm. B.P., mean blood pressure; Na₂CO₃, intravenous injection of 25 cc. 0.5 m sodium carbonate; A., arterial blood acidity curve; V., jugular blood acidity curve; C.S.F., cerebro-spinal fluid acidity curve; P.V., pulmonary ventilation; T., time in 6-second intervals.

usually the electrodes last several hours and give excellent records. A slight amount of protein from exceedingly small hemorrhage into the fourth ventricle seems to prolong the life of the electrode. Figure 12 shows a good behavior of the electrode and figure 10 a rapid drift. The rapid drift and disappearance of the manganese dioxide may be due to an abundance of reducing substances in the cerebro-spinal fluid. The protective action of blood is not so clear.
and increased pulmonary ventilation. The results are comparable to those shown in figure 8, where the electrode was placed between the cerebral convolutions.

Figure 11 shows the effects of intravenous injection of sodium carbonate: increased alkalinity of the arterial and venous blood and cerebro-spinal fluid. Accompanying these changes there is a decrease in respiration and a fall in blood pressure. Assuming for the moment that the behavior of cerebro-spinal fluid represents the changes in pH of the tissues, the explanation of the associated respiratory phenomena on an acid basis is supported.

In figure 12 the effects of intravenous injection of sodium bicarbonate are shown. There is an increased alkalinity of the arterial and venous blood, and increased acidity of the cerebro-spinal fluid. The alkalinity of the arterial and venous blood is long maintained and shows very little tendency to recover to its original level, but despite this changed reaction the cerebro-spinal fluid acidity curve holds almost horizontal at its new acid level. At the end of ten minutes it is still distinctly more acid than normal. Associated with this increased acidity there is a prolonged increase in blood pressure and an increase in pulmonary ventilation outlasting the duration of the published record. The close parallelism between the circulatory and respiratory response and the acidity of the cerebro-spinal fluid cannot but support the previous suggestion of a causal relation between the two phenomena (3).

Theoretically, at least, the intravenous injection of sodium bicarbonate simply by increasing the concentration of CO₂, H₂CO₃, and HCO₃⁻ ions in the blood prevents the diffusion outwards of the CO₂, H₂CO₃, and the HCO₃⁻ to which the H⁺ ion is attached. The acid formed in the tissues should, therefore, accumulate. If this is true it is a principle of utmost importance in the physiology of respiration.
The significance of such findings applied to tissue acidity is too great to omit a check on the acidity changes occurring within the cerebro-spinal fluid itself. Previous checks with the hydrogen and quinhydrone electrode have demonstrated the value of the data supplied by the manganese dioxide electrode in the circulating blood. Similar checks have been repeated on the cerebro-spinal fluid. By inserting a sampling tube into the fourth ventricle, small samples (0.2 cc) of cerebro-spinal fluid were withdrawn and the pH value determined with the quinhydrone electrode. A comparison of the curves with the graphic records obtained with the manganese dioxide electrode is shown in figure 13: record A—the administration of carbon dioxide, records B and C—the injection of sodium carbonate, records D and E—the injection of sodium bicarbonate. The curves show directional agreement throughout with the exception of
one observation in record D, in which the manganese dioxide curve shows an extra acid swing. For some unknown reason this effect occasionally resulted from drawing a sample.

Quantitative agreement is not indicated in the figures but as no effort was made to determine the E. M. F. changes per 0.1 pH in cerebro-spinal fluid in vitro experiments quantitative comparison is impossible. The chief concern at this stage of the problem was to establish whether or not the manganese dioxide electrode is a reliable indicator of directional changes in acidity in the cerebro-spinal fluid. Unfortunately we cannot be too certain of the validity of checks with the quinhydrone electrode for as was pointed out before the potential of both electrodes is affected by reducing substances, but inasmuch as it was necessary to remove very small samples of cerebro-spinal fluid the quinhydrone electrode was adhered to. As will appear below there were no indications of altered metabolism which might vary the amount of reducing substance. All that can be said at present is that the check with the quinhydrone electrode confirms the manganese dioxide electrode.

Accepting the results as valid, are we justified in accepting the proposed explanation of the acid effects of bicarbonate (1) (3) (5) based on the relative shift of acid and base determined by the rate of diffusion of and the impermeability of the membrane to the anions and cations of a bicarbonate buffer mixture? It is desirable to rule out other possible factors which might account for the increased acidity of the cerebro-spinal fluid such as circulatory disturbances and basal metabolism. It is hard to see how circulation of the blood could account for the increased acidity of the cerebro-spinal fluid, for Doctor Bronk and one of us have shown (unpublished work with the continuous thermophile method (8) of recording volume flow of blood) that the carotid flow of blood is enormously increased by the injection of bicarbonate. Neither are there indications that changes in basal metabolism (see records) could account for the acid changes in the cerebro-spinal fluid. In addition—unpublished experiments by Dr. McGinty and one of us, with the continuous method of recording changes in expired carbon dioxide and oxygen, show an increased elimination of carbon dioxide through the lungs on the administration of sodium bicarbonate. It is possible that sodium bicarbonate acts in the same way in the lungs and tissues in expelling acid from the blood. We are for the present forced back to the effects of sodium bicarbonate on the buffer mixtures in the blood and on the impermeability of membranes. Certainly figure 12 suggests a high degree of impermeability to base of the membranes determining the composition of the cerebro-spinal fluid. The prolonged maintenance of the increased acidity of the cerebro-spinal fluid despite the maintained alkalinity of the blood is strongly supporting evidence. On the other hand, if conditions existed for the passage of both
carbon dioxide and sodium bicarbonate into the cerebro-spinal fluid but in
an acid ratio, for example, greater than \( \frac{1}{20} \), the cerebro-spinal fluid would
turn acid too. Such a movement of acid and base is not inconceivable.
As a matter of fact it has been demonstrated in the stratification experi-
ments without the participation of a membrane. The same condition
might result in the cerebro-spinal fluid and would also be analogous to the
conditions which appear to exist at the arterial electrode immediately
following an injection of sodium bicarbonate (see fig. 12). There is an
indication of initial increased acidity possibly resulting from the advance
migration of acid followed by a reverse deflection possibly due to increased
alkalinity resulting from the arrival of the more slowly moving sodium at
the electrode. But the prolonged change in reaction of both the cerebro-
spinal fluid and blood in figure 12, as contrasted with the momentary
changes at the electrode, speak against this explanation.

Other evidence indicating the significance of impermeability is found in
the effects of sodium carbonate. Not infrequently sodium carbonate
exerts both alkaline and acid effects upon the cerebro-spinal fluid. An
initial alkaline effect associated with decreased blood pressure and pul-
monary ventilation is followed in several minutes by increased acidity and
increased ventilation and blood pressure above the normal values. Pro-
vided the membranes involved possess a high degree of impermeability to
base, the initial alkaline effect is explainable by the extraction of carbon
dioxide from the tissues into the blood. But once the carbonate has
changed to bicarbonate and free carbon dioxide again begins to accumulate,
the back pressure produced by the increased bicarbonate should lead to
further accumulation of acid in the tissues. The fact that carbonate exerts
this acid effect after a lapse of three to five minutes, during which time
base might conceivably enter the tissues very freely were the membranes
permeable to base, suggests a high degree of impermeability to sodium.

The degree of impermeability, however, seems to vary. With very large
injections of sodium bicarbonate corresponding large increases in acidity
of the cerebro-spinal fluid occur—sometimes as large as 0.2 to 0.3 pH—but
after the initial increase in acidity a relatively rapid alkaline drift
occurs. This drift might be explainable either by a parallel elimination
of bicarbonate by the kidneys, which would decrease the back pressure of
the sodium bicarbonate, or by an increased permeability of the cerebral
membranes. We have shown a rapid elimination of alkali by the kidneys
following the injection of sodium carbonate (9). On the other hand, an
initial alkalinity of the cerebro-spinal fluid, following the administration
of sodium bicarbonate, as shown in figure 14, suggests, in that particular
experiment, a free movement of base through a freely permeable membrane.
The fact that such results are obtained more frequently toward the close of
an experiment or after severe abuse, such as the administration of low
oxygen or sodium cyanide, points to injury of the membranes. It is interesting to note that when sodium bicarbonate produces these alkaline changes in the cerebro-spinal fluid it likewise depresses respiration and blood pressure.

Occasionally the administration of sodium bicarbonate may have no effect whatever on pulmonary ventilation. Is this due to a passage of acid and base in a non-stimulating ratio? Obviously this phase of the subject requires further critical experiments for an ultimate understanding of the effects of sodium bicarbonate. Chemical analyses of the cerebro-spinal fluid are desirable. Some have been made but not to our entire satisfaction. The difficulties opposing clean cut observations though perhaps not insurmountable are considerable. Such results as have been obtained adapt themselves to the views so far expressed: for example, a decreased carbon dioxide content of the cerebro-spinal fluid following the administration of sodium carbonate has been found which indicates high degree of impermeability, and also increased carbon dioxide content during the alkaline drift following the increased acidity of the cerebro-spinal fluid elicited by injection of sodium bicarbonate which indicates abnormal permeability. We hope to have more complete chemical data in the future but for the present we are inclined to believe that the data at hand suggest that the behavior of the membrane plays an important part in explaining the effects of sodium bicarbonate.

Discussion. As the logical method of presentation was used there is little need for further discussion. There is one point, however, which calls for more attention, namely, to what extent are we justified in assuming that changes in acidity of the cerebro-spinal fluid reflect the changes in acidity of the respiratory center. There is no reason for believing that the acidity

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Fig. 14. Observation showing alkaline effect of the intravenous injection of sodium bicarbonate on the cerebro-spinal fluid. B.P., mean blood pressure; C.S.F., cerebro-spinal fluid acidity curve; A., carotid blood acidity curve; V., jugular blood acidity curve; P.V., pulmonary ventilation.
of the cerebro-spinal fluid is an infallible index to the acidity of the respiratory center.

In evaluating the significance of the acidity of the cerebro-spinal fluid with respect to the acidity of the brain, it should be recognized that the cerebro-spinal fluid is subjected to several influences which may affect its acidity. Not only is it affected by the permeability of membranes, but by the metabolism of the nerve cells which determines the acid contribution of the brain to the fluid and the ventilating effect of the choroid plexus which determines the amount of acid brought to and taken away from the cerebro-spinal fluid.

In these experiments there are no obvious reasons for believing that altered metabolism plays a rôle in the interpretation of results. On the other hand the increased blood flow through the brain on the administration of carbon dioxide exerts a ventilating effect in bringing large amounts of acid to the brain and the increased flow of blood on the administration of sodium carbonate improves ventilation by increasing the transport of acid away from the cerebro-spinal fluid. But sodium bicarbonate also increases the flow of blood. What then is the ventilating effect in this instance? Obviously if increased flow of blood were the only effect of sodium bicarbonate the cerebro-spinal fluid should turn alkaline. In general, the extent of the ventilating effect of the choroid plexus is determined by the flow of blood. The directional effect is determined by the composition of the blood.

If this logic is correct there is reason for believing that in these particular experiments the changes in acidity of the cerebro-spinal fluid is a fair index to the changes in acidity of the respiratory center. But differences are not inconceivable. There is no reason for believing that the membranes involved in functional activity of the respiratory center are identical in behavior to the membranes responsible for the composition of the cerebro-spinal fluid. But on the whole—the agreement between pulmonary ventilation and acidity of the cerebro-spinal fluid is good.

**SUMMARY**

The chemical regulation of respiration was studied with the continuous method of recording changes in acidity in the circulating blood and body fluids.

The relation of respiration to the acidity of the respiratory center was analyzed by a study of the changes in acidity of the arterial and venous blood and the cerebro-spinal fluid.

Comparison of the arterial and venous blood acidity curves gave information on the passage of acid and base between blood and tissue. It indicated the extent of buffering by the tissues and the consequent changes of acidity in the tissues. Synchronous records of the acidity of the cerebro-spinal
fluid gave collateral evidence on the effects of semipermeability of membranes on acid-base equilibrium and tissue acidity.

The administration of carbon dioxide increased the acidity of the arterial and venous blood. The changes in acidity were greater and more abrupt on the arterial side of the tissues than on the venous side. This suggested a passage of carbon dioxide from the blood into the tissues during the administration of carbon dioxide and a passage from the tissues into the blood during recovery. The tissues accordingly acted as buffers to the blood and in the process became more acid. The increased acidity of the tissues was accompanied by increased pulmonary ventilation.

The intravenous injection of sodium carbonate increased the alkalinity of the arterial and venous blood. The changes in alkalinity were greater and more abrupt on the arterial side of the tissues than on the venous side. The hampered movement of base suggested a passage of carbon dioxide from the tissues into the blood on injection and retention of carbon dioxide by the tissues during recovery. The tissues accordingly acted as buffers to the blood and in the process became more alkaline. The increased alkalinity of the tissues was accompanied by decreased pulmonary ventilation and blood pressure.

The intravenous injection of sodium bicarbonate increased the alkalinity of the arterial and venous blood but the changes in arterial and venous alkalinity were frequently almost identical. This suggested smaller exchange between blood and tissue and a smaller buffering effect of the tissues on the blood. The tendency of the tissues to become alkaline on the injection of sodium bicarbonate therefore seemed to be missing. Moreover, increased respiration and blood pressure commonly occurred suggesting that sodium bicarbonate increased the acidity of the tissues.

The experiments in which the electrode was placed in tissue gave more direct information on the changes occurring in the tissues. The manganese dioxide electrode inserted between the muscle fibers of the sartorius muscle showed increased acidity on the administration of carbon dioxide. On the intravenous injection of sodium carbonate the electrode showed increased alkalinity. On the intravenous injection of sodium bicarbonate the electrode showed increased acidity. These results seemed explainable on the effect of relative impermeability of membranes to base.

This effect of impermeability of membranes was studied further by recording changes in acidity of the cerebro-spinal fluid. The administration of carbon dioxide increased the acidity of the arterial and venous blood and cerebro-spinal fluid. Intravenous injection of sodium carbonate increased the alkalinity of the arterial and venous blood and cerebro-spinal fluid. Intravenous injection of sodium bicarbonate increased the alkalinity of the arterial and venous blood and the acidity of the cerebro-spinal fluid. The increased acidity of the cerebro-spinal fluid was main-
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...tained despite a prolonged increase in alkalinity of the blood. Such findings indicated a high degree of impermeability of the cerebral membranes to base. Assuming that the membranes of or about the respiratory center function in an analogous way the acidity of the cerebro-spinal fluid serves as an index to the acidity of the respiratory center.

Towards the close of an experiment or after severe abuse entailed by the administration of low oxygen or sodium cyanide the injection of sodium bicarbonate increased the alkalinity of the cerebro-spinal fluid. It was suggested that such results are due to impaired impermeability of the cerebral membranes permitting the passage of base from the blood into the cerebro-spinal fluid. When the injection of sodium bicarbonate increased the alkalinity of the cerebro-spinal fluid it decreased respiration and mean blood pressure.

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