THE REGULATION OF RESPIRATION

III. A CONTINUOUS METHOD OF RECORDING CHANGES IN ACIDITY APPLIED TO THE CIRCULATING BLOOD AND OTHER BODY FLUIDS

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The importance of a constant hydrogen ion concentration of the arterial blood to normal tissue function has been greatly stressed within the past decade. The apparent dependency of tissue acidity on blood acidity coupled with the constancy of the arterial hydrogen ion concentration in the normal individual seemed to justify this emphasis. This constancy of hydrogen ion concentration of the arterial blood was attributed to the extreme sensitivity of the respiratory center to the free hydrogen ion of the blood. In fact this view became so firmly established that the absence of a demonstrable increased acidity of the arterial blood during hyperpnea was considered indicative of the supersensitivity of the respiratory center to the present physico-chemical methods of detecting the hydrogen ion. It is, therefore, not surprising that the technique for the determination of the hydrogen ion concentration of the blood has advanced so rapidly within the past few years. It seems now, however, that the problem of respiratory control must be considered from a somewhat different angle. Direct experiments (1) and the review of extensive literature (2) indicate a gross insensitivity of the respiratory mechanism to the free hydrogen ion of the arterial blood. Indeed, the relatively greater frequency of the inverse relation of pulmonary ventilation to the hydrogen ion concentration of the blood indicates that a causal relationship is wanting. Hence a detailed elucidation of the relation of pulmonary ventilation to the hydrogen ion concentration of the blood seemed of sufficient interest to warrant an effort to develop a continuous method of recording changes in the hydrogen ion concentration of the circulating blood. The advantages of a continuous method in determining the time relation of acidity changes to

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pulmonary ventilation, oxygen consumption, blood pressure, heart rate, etc.; the possibility of recording synchronously changes in acidity of the arterial and venous blood, cerebro-spinal fluid (3), urine (4), and other body fluids; the large amount of data obtainable as compared with the discontinuous method of sampling—offer invaluable opportunities for advancing the subjects of respiratory control and acid-base equilibrium.

METHODS. The experiments of Roaf (5) on the time relation of acid production in muscle during muscle contraction suggested the use of the manganese dioxide electrode studied by Tower (6) and Smith (7). The electrode has two advantages which particularly recommend its use. As it functions independently of the pressures of hydrogen and oxygen it excludes the necessity of saturation of the blood with hydrogen, and permits the recording of changes in blood acidity in the presence of synchronous changes in oxygen pressure of the blood. On the other hand, it is stated that the absolute values obtained with the electrode are unreliable. This may appear to be a serious objection, but we believe that the short observations of the acute experiments showing rapid changes in blood acidity and pulmonary ventilation may, for a time at least, lead to more rapid progress in the study of respiratory control than the accurate determination of the hydrogen ion concentration of the blood in long-established conditions of equilibrium. Provided the electrode gives significant information on the magnitude and direction of changes in acidity it should prove to be a valuable tool.

The development of the use of the manganese dioxide electrode resolved itself into several distinct problems: the mechanical registration of changes in acidity, the preparation of a suitable electrode, the establishment of the validity of data yielded by the electrode, and the application of the method to the circulating blood and other body fluids of the living animal. The difficulties were varied and numerous, some are still to be overcome. Inasmuch as the behavior of the manganese dioxide electrode, especially in complex fluids, is imperfectly understood many of our difficulties were solved by purely empirical methods of trial and error; but by gradual elimination we have adopted methods which yield significant data.

In vitro experiments. The manganese dioxide electrodes were placed in the stream of fluids studied. The fluids were kept at a constant rate of flow by means of a mechanically operated syringe of 100 cc. capacity and a three-way stop cock, which allowed an alternate change of fluids of different pH values. The potentiometer circuit was closed with a saturated potassium chloride calomel electrode, as shown in figures 1 and 2. By using four potentiometers (the type K Leeds & Northrup potentiometer proved a very satisfactory instrument) it was possible to obtain four synchronous records and thus test for the reproducibility of behavior of electrodes under
similar conditions. Permanent records of changes in acidity were made on smoked paper with the mechanical method employed by one of us in registering the electrical deflections of the submaxillary gland (8)—the method differing in that the galvanometer was maintained at zero and the compensating E.M.F. recorded by vertical writing points attached by thread to spindles mounted on the potentiometer drum. The spindles were of 1.655 cm. diameter, which gave a vertical deflection of 52.0 mm. per 0.01 volt change in E.M.F. The smoked paper accommodated changes in E.M.F. of approximately 0.05 volt. The initial level of the record was conveniently placed at any height on the drum by a ratchet device on the spindle, one complete turn representing 0.01 volt. Not infrequently the

Fig. 1. In vivo method for recording changes in acidity of the blood with the use of sodium oxalate as an anticoagulant.

initial position of the drum preceding an observation may be at its upper limit (lowest E.M.F.) or at its lowest position (highest E.M.F.). If the change in pH is in the alkaline direction the first position is awkward for registering the change, and if in the acid direction the second position is equally inconvenient. Either position requires an adjustment of the coarse resistance followed by a complete shift of ten revolutions of the sliding contact. To avoid this inconvenience and loss of continuity of the record a 5 ohm resistance was placed in parallel with one of the 5 ohm resistance coils of the coarse adjustment and the working current readjusted. This
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brings the sliding contact to the mid position which is favorable for registering changes in acidity in either direction.

The ideal electrode should possess the properties of sensitivity, rapidity of response, sturdiness and reproducibility. It soon became apparent that these conditions were hard to meet. The thinly plated electrode though sensitive and rapid in response was quick to dissolve and, therefore, unsatisfactory for prolonged experiments. The thickly coated electrode was found to possess durability but was sluggish in response and less sensitive to changes in acidity. A compromise was reached between durability on the one hand and sensitivity and quickness of response on the other by adjusting the duration of plating. Reproducibility was accomplished by following a standard routine of preparation. The following procedure was adopted.

A piece of no. 24 platinum wire about 6 to 8 mm. long was sealed into the end of a glass tube 2 mm. inside diameter, 3 mm. outside diameter, and 7 cm. long. The protruding end, about 1 mm. in length, was rounded on a fine stone (to avoid point effects) then heavily plated with platinum black and heated to red heat in the alcohol flame. This electrode connected with the positive pole of a 6 volt battery was plated for one and one-half minutes in acidified \( \text{H}_2\text{SO}_4 \) 0.4 N solution of manganese sulphate with 650 ohms resistance in the external circuit. The negative electrode of similar construction was placed 2 cm. from the positive electrode. A 2 N manganese sulphate solution which served as a stock solution was freshly prepared every seven days. To avoid the annoyance of chance poisoning of the platinum, new platinum was used for each electrode. We are not certain that these precautions are necessary, but scrupulous cleanliness and the use of fresh materials have materially decreased irregularity in behavior of the electrodes.

Since the freshly prepared electrodes show an alkaline drift—rapid at first but gradually diminishing in rate—they were equilibrated in the fluids tested. It appears that complete equilibration was seldom obtained. In phosphate buffer mixtures the drift was rapid and mostly over within fifteen to thirty minutes. In egg albumen, blood plasma, and whole blood a slow drift persisted for several hours. The electrodes were, therefore, equilibrated for three or four hours before using. It seems probable that the more prolonged drift in protein solution is associated with the formation
of a protein film on the electrode. Drift obviously is an important factor in reproducibility of absolute E.M.F. values for a given pH.

Tower reports a high grade of reproducibility of electrodes with variations in absolute E.M.F. values amounting only to 0.01-0.02 pH. With the use of the formula \( \pi = \frac{RT}{\mu} \ln \frac{C_{\text{Mn}} C_{\text{H}^{+}}}{C_{\text{Mn}'} C_{\text{H}^{+}'}^{2.56}} \) he determined the pH values. Smith, on the other hand, was unsuccessful in obtaining the same reproducibility of results—particularly with a group of acids designated “inconstant;” and with the “constant” group of acids he found that \( \pi = \frac{RT}{\mu} \ln \frac{C_{\text{Mn}} C_{\text{H}^{+}p}}{C_{\text{Mn}'} C_{\text{H}^{+}'p}^{2.56}} \) agreed better with his observations.

Inasmuch as \( \pi \) is determined not only by the concentration of the H ions but by the Mn ions as well, and as we made no effort to control the concentration of Mn ions, we could hardly expect to reproduce the absolute E.M.F. values of Tower and Smith even though we had confined our experiments to the simple acid solutions which they employed. Nevertheless some of the results which we have obtained may be of interest in indicating the behavior of the electrode—particularly in complex organic solutions. The data in table 1 are collected from experiments on blood plasma, egg albumen, and phosphate mixtures. The third column gives the pH values of the fluids determined with either the hydrogen or the quinhydrone electrode (9). The fourth column gives the corresponding E.M.F. values observed with the manganese dioxide electrode. Column five gives the corrected E.M.F. values for pH 7.4. Column six gives the drift in volts per hour.

For a pH value 7.4 the table shows absolute E.M.F. values ranging from 0.2547 to 0.4809 volts—a difference in terms of pH of approximately 2.3. The explanation of this enormous difference is uncertain but it probably is not due to drift. Inasmuch as the electrodes were uniformly treated (two hours' equilibration in a stationary vessel followed by one and one-half

\[ \text{RT} \ln \frac{C_{\text{Mn}} C_{\text{H}^{+}}}{C_{\text{Mn}'} C_{\text{H}^{+}'}^{2.56}} \]  

2 The existence of such a film may be demonstrated by the electrolytic evolution of hydrogen or oxygen on the electrode, when the gas bubbles cause the separation of a gray film from the surface of the electrode. This is further demonstrated in an experiment with two egg albumen solutions of the same pH but different NaCl concentrations. The direction of E.M.F. change of the MnO₂ electrode on changing from one of these solutions to the other, may be predicted from the difference in NaCl concentration. The effect, however, is not large and is temporary. The MnO₂ electrode soon comes back to its original E.M.F. This effect can be obtained only with fairly large variation in salt concentration and is probably of no importance in the application of the electrode to body fluids. It, however, probably accounts for the irregularities in behavior of the MnO₂ electrode associated with changes in arterial blood pressure—irregularities that were observed when we used the electrode in the blood-stream, employing sodium oxalate an an anticoagulant.
TABLE 1
Data from in vitro experiments showing E.M.F. values for pH 7.4

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>FLUID USED</th>
<th>OBSERVED pH WITH (H) HYDROGEN ELECTRODE OR (QH) QUINHIDRON ELECTRODE</th>
<th>OBSERVED E.M.F. WITH MnO₂ ELECTRODE</th>
<th>MnO₂ E.M.F. VALUES CORRECTED FOR pH OF 7.4</th>
<th>DRIFT IN VOLTS PER HOUR EQUILIBRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Plasma</td>
<td>H 7.534</td>
<td>0.2557</td>
<td>0.2547</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Plasma</td>
<td>QH 7.605</td>
<td>0.2503 0.2734</td>
<td>0.0046</td>
<td>0.0037</td>
</tr>
<tr>
<td>14</td>
<td>Plasma</td>
<td>QH 7.574</td>
<td>0.2506 0.2737</td>
<td>0.0042</td>
<td>0.0053</td>
</tr>
<tr>
<td>15</td>
<td>Plasma</td>
<td>QH 7.466</td>
<td>0.2497 0.2728</td>
<td>0.0053</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Plasma</td>
<td>QH 7.456</td>
<td>0.2555 0.2758</td>
<td>0.0029</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Egg albumen</td>
<td>QH 7.736</td>
<td>0.2548 0.2721</td>
<td>0.0037</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Phosphate</td>
<td>QH 7.217</td>
<td>0.2513 0.2686</td>
<td>0.0022</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Phosphate</td>
<td>QH 7.237</td>
<td>0.2476 0.2649</td>
<td>0.0027</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Plasma</td>
<td>H 7.534</td>
<td>0.2500 0.2734</td>
<td>0.0046</td>
<td>0.0037</td>
</tr>
<tr>
<td>13</td>
<td>Plasma</td>
<td>QH 7.605</td>
<td>0.2500 0.2734</td>
<td>0.0046</td>
<td>0.0037</td>
</tr>
<tr>
<td>14</td>
<td>Plasma</td>
<td>QH 7.574</td>
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</tr>
<tr>
<td>15</td>
<td>Plasma</td>
<td>QH 7.466</td>
<td>0.2500 0.2734</td>
<td>0.0046</td>
<td>0.0037</td>
</tr>
<tr>
<td>16</td>
<td>Plasma</td>
<td>QH 7.456</td>
<td>0.2500 0.2734</td>
<td>0.0046</td>
<td>0.0037</td>
</tr>
</tbody>
</table>

* The data for computing E.M.F. values for pH 7.4 are found in the last column of table 2.
† Acid drifts. Probably due to increasing acidity from bacterial decomposition.
‡ Precipitation about electrode while plating.
### Table 2

Data from in vitro experiments showing E.M.F. changes of MnO₂ electrodes with changes in hydrogen ion concentration.

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>FLUID USED</th>
<th>OBSERVED pH WITH (H) HYDROGEN ELECTRODE OR (QH) QUINHYDROX ELECTRODES</th>
<th>pH CHANGE</th>
<th>NUMBER OF MnO₂ ELECTRODES</th>
<th>E.M.F. CHANGE OF MnO₂ FBR. 0.1 pH MV.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phosphate</td>
<td>H 7.2 - 7.4</td>
<td>0.20</td>
<td>1</td>
<td>8.55</td>
</tr>
<tr>
<td>2</td>
<td>Phosphate</td>
<td>H 7.321 - 7.492</td>
<td>0.171</td>
<td>3</td>
<td>9.10</td>
</tr>
<tr>
<td>3</td>
<td>Phosphate</td>
<td>QH 6.948 - 7.217</td>
<td>0.269</td>
<td>4</td>
<td>8.21</td>
</tr>
<tr>
<td>4</td>
<td>Phosphate</td>
<td>QH 6.969 - 7.237</td>
<td>0.268</td>
<td>4</td>
<td>8.12</td>
</tr>
<tr>
<td>5</td>
<td>Phosphate</td>
<td>QH 6.858 - 7.237</td>
<td>0.379</td>
<td>4</td>
<td>8.17</td>
</tr>
<tr>
<td>6</td>
<td>Egg albumin</td>
<td>H 7.546 - 8.355</td>
<td>0.809</td>
<td>1</td>
<td>8.00</td>
</tr>
<tr>
<td>7</td>
<td>Egg albumin</td>
<td>H 6.98 - 7.123</td>
<td>0.143</td>
<td>2</td>
<td>8.07</td>
</tr>
<tr>
<td>8</td>
<td>Egg albumin</td>
<td>H 6.767 - 6.869</td>
<td>0.102</td>
<td>1</td>
<td>8.07</td>
</tr>
<tr>
<td>9</td>
<td>Egg albumin</td>
<td>H 6.764 - 7.124</td>
<td>0.360</td>
<td>1</td>
<td>8.07</td>
</tr>
<tr>
<td>10</td>
<td>Egg albumin</td>
<td>H 7.739 - 7.881</td>
<td>0.142</td>
<td>1</td>
<td>8.07</td>
</tr>
<tr>
<td>11</td>
<td>Egg albumin</td>
<td>QH 6.428 - 6.736</td>
<td>0.318</td>
<td>4</td>
<td>8.07</td>
</tr>
<tr>
<td>12</td>
<td>Plasma</td>
<td>H 7.743 - 7.534</td>
<td>0.209</td>
<td>2</td>
<td>8.07</td>
</tr>
<tr>
<td>13</td>
<td>Plasma</td>
<td>QH 7.005 - 7.782</td>
<td>0.177</td>
<td>4</td>
<td>8.07</td>
</tr>
</tbody>
</table>
CONTINUOUS RECORDS OF CHANGES IN BLOOD ACIDITY

TABLE 2—Concluded

<table>
<thead>
<tr>
<th>EXPERTISE</th>
<th>FLUID USED</th>
<th>OBSERVED pH WITH (H) HYDROGEN ELECTRODE OR (QH) QUINHYDRONE ELECTRODE</th>
<th>pH CHANGE</th>
<th>NUMBER OF MnO2 ELECTRODES</th>
<th>E.M.F. CHANGE OF MnO2 PER 0.1 pH MV.</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Plasma</td>
<td>QH 7.574-7.776</td>
<td>0.202</td>
<td>4</td>
<td>9.59, 9.74, 10.20, 10.24</td>
</tr>
<tr>
<td>15</td>
<td>Plasma</td>
<td>QH 7.194-7.466</td>
<td>0.272</td>
<td>4</td>
<td>12.13, 12.17, 12.44, 12.62</td>
</tr>
<tr>
<td>16</td>
<td>Plasma</td>
<td>QH 6.716-7.458</td>
<td>0.740</td>
<td>4</td>
<td>10.92, 10.97, 11.57, 11.74</td>
</tr>
</tbody>
</table>

TABLE 3
Summary of results of in vitro calibration experiments

<table>
<thead>
<tr>
<th>pH DETERMINED BY</th>
<th>IN PHOSPHATE</th>
<th>IN EGG ALBUMIN</th>
<th>IN PLASMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.M.F. change of MnO2 electrode per 0.1 pH mv.</td>
<td>Number of experiments</td>
<td>E.M.F. change of MnO2 electrode per 0.1 pH mv.</td>
</tr>
<tr>
<td>H2 electrode</td>
<td>8.60 (8.20-9.10)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Quinhydrone</td>
<td>8.12 (7.97-8.47)</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Average</td>
<td>8.23</td>
<td>8.33</td>
<td>10.87</td>
</tr>
</tbody>
</table>

hours in a rocking vessel), and inasmuch as the drift following such equilibration was at most 0.005 volt per hour—the main difference in E.M.F. values must be due to some other factor. This factor may be a specific reducing effect of the fluid though this is a point which we have not studied in detail and, therefore, do not care to press. It is suggested, however, as an explanation of the difference in E.M.F. values found in the blood plasmas of different animals.

The maximum difference in blood plasmas in our experiments amounted to 0.0446 volt or approximately 0.45 pH. Such differences in biological
work constitute a gross error, but as our main interest is to study changes in pH this variability in absolute values loses significance and especially so if the base line for any particular observation is set by a single determination with methods yielding absolute values. The question of greater concern is the reproducibility of the behavior of electrodes under similar conditions. This reproducibility of behavior is best demonstrated by running several freshly prepared and equilibrated electrodes at the same time in the same fluid. For reproducibility of absolute values under such conditions, see table 1. Note experiment 13, in which absolute values obtained with four electrodes checked within 0.03 pH. Note also the uniformity of drift of approximately 0.04 pH per hour for each electrode. Such uniformity of behavior of electrodes is highly desirable particularly for quantitative comparison of synchronous changes in acidity in the arterial and venous blood, the cerebro-spinal and other body fluids.

For reproducibility of behavior to changes in pH in similar and dissimilar fluids, see tables 2 and 3 and figure 3. Figure 3 shows simultaneous records obtained with four electrodes in the stream of plasmas with alternating pH values of 7.605 and 7.782. The curves are mutually superimpossible despite the alkaline drift. Compared with other methods of determining acidity the records obtained with the manganese dioxide electrode show a degree of uniformity which one could hardly hope to duplicate with any discontinuous method. Figure 4 from an in vivo experiment shows the parallelism in behavior of two electrodes one in each of the carotid blood-streams. We have observed such reproducibility so often that we feel that our results on the arterial and venous bloods are comparable on a qualitative basis.

Though there is uniformity in behavior of similar electrodes in similar fluids to changes in pH the behavior in dissimilar fluids is variable. This is shown in tables 2 and 3, in which the results of sixteen experiments (using forty-four electrodes) on phosphate mixtures, egg albumen and blood plasmas are given. Column two gives the fluid studied. Column three gives the pH ranges of the fluids used, as determined with the hydrogen and quinhydrone electrodes. Column four gives the pH change, and column five the E.M.F. change in mv. per 0.1 pH. The results are summarized in table 3. The sensitivity of the electrodes varied from 0.19 to 12.62 mv. per 0.1 pH. The lowest sensitivity of the electrode was observed in the phosphate mixtures with an average change in E.M.F. of 8.23 mv. per 0.1 pH, and the highest sensitivity in blood plasma with an average change of 10.87 mv. per 0.1 pH. In egg albumin solutions the electrode showed an intermediate sensitivity of 8.33 mv. per 0.1 pH.

In vivo experiments. Two distinctly different methods were used in the animal experiments. The first method, shown in figure 1, has been abandoned for simpler arrangements, but since it eliminates the employ-
Fig. 3. Smoked record showing changes in acidity synchronously recorded by four manganese dioxide electrodes in the in vitro experiment. Downstroke indicates decreased acidity.

Fig. 4. In vivo experiment showing changes in acidity in the circulating blood of the left and right carotid arteries and left external jugular vein on the administration of carbon dioxide and the intravenous injection of sodium bicarbonate. The similarity of the acidity curves in the carotid arteries demonstrates the reproducibility of behavior of electrodes in the in-vivo experiment.
ment of expensive anticoagulants, and with certain precautions yields reliable data it is briefly described. A specially constructed electrode vessel with a central tube extended in the form of a cannula surrounded by a water jacket served to bring the blood at body temperature to the electrode. With an electrically driven syringe of 100 cc. capacity the blood was drawn from either artery or vein past the electrode at a constant rate of about 100 cc. in forty minutes. Coagulation of the blood was prevented by M/6 sodium oxalate solution delivered at body temperature through a fine capillary tube running through the central tube of the electrode vessel to the very tip of the cannula, insuring a mixture of the blood with the oxalate solution immediately upon leaving its natural vessel. The oxalate solution flowed at about one-tenth the rate of the syringe flow. To prevent unevenness of the oxalate flow due to the oscillations in blood pressure the reservoir of oxalate solution was raised well above the animal (8 meters). Later a small syringe operated in unison with the blood syringe served to supply the oxalate. This arrangement gave smooth records with the arterial blood but the venous records were decidedly irregular. The irregularity proved to be due to uneven mixing of the blood and oxalate solution, and was overcome by an electrically operated pulsator. The pulsator tapping the rubber tube leaving the electrode vessel sent a series of waves into the vein which produced a thorough mixing of the oxalate solution and blood before they reached the electrode. The toxicity of the oxalated blood prevented its reinjection. To delay the harmful effects of loss of blood large dogs were used and the blood replaced with blood substitutes.

It was the inability to obtain hirudin that led to the development of this method, and initial failure with heparin to its continuance. On the venous side the method apparently yielded reliable data under all conditions but on the arterial side changes in blood pressure were accompanied by changes in E.M.F. An increase in blood pressure was associated with an apparent increase in acidity and the reverse held for a fall in blood pressure whether the pressure changed on the administration of CO₂ or mechanical pressure on the abdomen. These effects of blood pressure proved to be due to changes in the proportion of oxalate solution and blood reaching the electrode. The difficulty of correcting this disturbance, which involves the elimination of stretch of the electrode vessel and its connecting tubes, led to another trial of heparin, which proved successful.

The electrode was placed in the natural course of the blood of the heparinized dog. A simple combination electrode and blood sampling cannula of the design shown in figure 2 was inserted, like the ordinary T-shaped cannula, into either the carotid artery or external jugular vein. A calomel electrode was connected with the left tube, the manganese dioxide electrode inserted into the central tube, and blood samples drawn from the right
Continuous records of changes in blood acidity

The blood sampling tube was of barometer bore, provided with a two-way stopcock and sealed with a heavy rubber membrane. By piercing the rubber membrane first, then opening the cock and inserting the syringe needle directly into the blood stream, and similarly withdrawing the needle in two stages blood samples were taken without extra loss of blood. Operative preparations were made with a thermo-cautery knife to minimize the oozing of blood following the administration of heparin. The femoral arteries were used to register mean blood pressure and for hemorrhage, the left femoral vein for intravenous injection, and the trachea for recording respiration and for the administration of gases with the rebreathing device. To insure a free flow of blood past the electrode, the electrode vessels were carefully aligned and rigidly fixed in the natural position of the vein and artery. This is particularly necessary for the venous electrode and as a further precaution the opposite external jugular vein was tied to shunt its flow of blood through the remaining patent veins. The electrode vessels were inserted last, followed immediately by the intravenous injection of heparin. The amount injected varied with the strength of the preparation. A few preparations were exceedingly weak. Injection of a gram or more failed to prevent clotting. Fortunately this valuable preparation appears to be improved with the use of newer methods of manufacture. At least the last three lots have been entirely satisfactory.

Intravenous injection of 25 to 30 mgm. per kilogram of body weight sufficed to prevent clotting and the collection of fibrin on the electrode for a period of four or five hours. No harmful effects from the administration of heparin have been observed.

Though the in vitro experiments indicated the reliability of the electrode for our problem, the final test of the use of the electrode in body fluids of the living animal rests on checks with standard methods of hydrogen ion determinations. These checks have been made both with the hydrogen electrode and with the quinhydrone electrode (9) in a number of procedures employed to study the chemical regulation of respiration.

Early in the animal experiments with the use of the hydrogen electrode we had satisfied ourselves of the value of the manganese dioxide elec-
trode in such procedures as the administration of carbon dioxide, sodium carbonate, ammonium chloride, and mechanical asphyxia. A comparison of the pH curves, obtained with the hydrogen electrode on blood samples

drawn at regular intervals from the femoral artery, with the continuous curve obtained with the manganese dioxide electrode is shown in figure 5. In transcribing the manganese dioxide acidity curve from the smoked record 10 mv. is taken to represent 0.1 pH. This graph is representative

Fig. 6. In vivo experiment showing effects of rapid injection of lactic acid on arterial pH. A comparison of the manganese dioxide acidity curve with the curve established by the quinhydrone electrode on individual blood samples.

Fig. 7. Changes in arterial pH following intravenous injection of 12 cc. 10 per cent NH₄Cl. Injection at arrow.

Fig. 8. Changes in venous pH following hemorrhage and subsequent injection of the blood: hemorrhage at 1, and injection at 2. Note the large alkaline drift of the manganese dioxide electrode which is corrected for in the middle dotted record.

Fig. 9. Changes in arterial pH with intravenous injection of 19 cc. M/2Na₂CO₃. Heavy bar = injection period.
of the agreement in all our experiments in which the hydrogen electrode was used.

More recently, with the application of the quinhydrone electrode to the determination of the hydrogen ion concentration of blood plasma, we have improved the checks on the behavior of the manganese dioxide electrode

![Fig. 10](image1)

**Fig. 10**

Changes in arterial pH with administration of nitrogen. Constant artificial respiration. Heavy bar — period of nitrogen administration.

**Fig. 11**

Changes in venous pH following intravenous injection of 30 cc. 10 per cent Na$_2$CO$_3$. Injection at start of record.

![Fig. 12](image2)

**Fig. 12**

Changes in arterial pH following intravenous injection of 8 cc. M/100 NaCN. Injection at arrow.

**Fig. 13**

Changes in pH of the venous blood with following procedures: at 1—breathing 20 per cent CO$_2$; at 2—injected 200 cc. 5 per cent sodium lactate (neutral); at 3 and 4—breathing 20 per cent CO$_2$. Note that MnO$_2$ values are at a somewhat more acid level at the end of the experiment than the quinhydrone values.

in the circulating blood. The quinhydrone electrode permits several determinations in a relatively short time on small samples of blood. It was, therefore, possible to establish a sufficient number of points on the quinhydrone acidity curve to permit a close comparison with the manganese dioxide acidity curve. All the data represented in the quinhydrone acidity
curves were obtained with the combination electrode and sampling vessel which were aligned to yield samples of blood which had just passed the electrode.  

The behavior of the manganese dioxide electrode, as checked with the quinhydrone electrode, is shown in figures 6 to 15, the effects of injection of lactic acid in figure 6, ammonium chloride in figure 7, hemorrhage in figure 8, intravenous injection of sodium carbonate in figures 9 and 10, and the administration of low oxygen in figure 11, mechanical asphyxia after the administration of room air and after pure oxygen in figure 12, the intravenous injection of sodium cyanide in figures 13 and 14, and sodium cyanide followed by sodium lactate and carbon dioxide in figure 15.

![Graph showing pH changes](http://ajplegacy.physiology.org/)

Fig. 14. Changes in pH of arterial blood following intravenous injection of sodium cyanide: at 1—injected 20 cc. M/100 NaCN; at 2—injected 33 cc. M/100 NaCN. Note agreement in pH values following first injection; disagreement following second injection.

These graphs were obtained from experiments on eight different animals and with eight different electrodes. In transcribing the manganese dioxide electrode curves 10 mv. is taken again to represent 0.1 pH (the reason for taking 10 mv. is explained below). In those experiments in which drift was very small no correction was made. In those in which drift was appreciable correction was made by determining the acid gradient over a relatively long period in which the condition of the animal was presumably

3 Clotting of the blood samples, which was likely to occur even though in vivo clotting was absent, interfered with the accuracy of pH determinations. This difficulty was avoided by drawing a drop of heparin solution in the syringe before taking the blood sample. Heating of the platinum electrode in the alcohol flame after each determination seemed to improve the accuracy of the method.
continuous records of changes in blood acidity

constant. Such corrections are seen in figure 8. It should be pointed out
that these corrections are subject to error. In the first place we do not
know why the drift varies in different experiments and, therefore, have no
means of knowing whether the conditions producing the drift are constant
throughout an experiment. On the whole the drift tends to disappear as
the experiment proceeds. This is likely to lead to over-correction. The
only safe procedure is to check the drift with other electrodes.

Other than this the graphs require little explanation. They present
the data in the simplest and clearest form. They show what may be
expected from the manganese dioxide electrodes under a variety of con-
ditions. On the whole there was very close agreement in configuration
of the curves. The main differences appear in the relative quickness of
the electrodes and the apparent difference in sensitivity. Seemingly less

pronounced sensitivity of the manganese dioxide electrode in the in vivo
experiments was undoubtedly due to the failure of the electrode to come
into complete equilibrium with the blood before a reverse change in acidity
occurred. We tried to correct for this factor in transcribing the man-
ganese dioxide acidity curve by taking a lower E.M.F. change per 0.1
pH than the average figures given in table 2. In some of the graphs this
correction satisfied the curves completely, in others not quite as perfectly,
but only in the observation on sodium cyanide is there a very obvious
discrepancy in results. It will be noted in figure 13 that despite the general
similarity in configuration of the curves the manganese dioxide electrode
was considerably slower. This appears again in the first observation in
figure 14. The second observation, on the effects of a considerably larger
injection of sodium cyanide, shows a greater discrepancy.

Fig. 15. Changes in pH of arterial blood with mechanical asphyxia. Solid bars
= duration of asphyxia: 1, 3, 6— asphyxia with room air: 2, 4, 8— asphyxia with
approximately 100 per cent oxygen.
We cannot connect this discrepancy in results with oxygen pressure. We have shown, in agreement with others, that in the in-vitro experiments oxygen exerted no effects on the behavior of the electrode. Alternate phosphate mixtures of the same pH but with 0 and 760 mm. oxygen pressure respectively developed the same E.M.F. The same held for plasma. Similarly in other in vitro experiments we found that the addition of cyanide to either phosphate mixtures or plasmas had no effect on the behavior of the electrode. Neither can the discrepancy of results be related to changes in the velocity flow of blood past the electrode for that factor proved to be of negligible importance. A large reduction in a rapid flow had no significant effect on the E.M.F. developed. A reduction from a relatively slow flow of plasma of 6.5 to 3.0 cc. per minute in the in vitro experiment was accompanied by an apparent increase of acidity of only 0.01 pH, and only when the flow was completely stopped was there a distinct change in E.M.F. in the acid direction amounting to approximately 0.03 or 0.04 pH.

Possibly the delayed response and alkaline values yielded by the manganese dioxide electrode following massive doses of cyanide was due to the formation of metabolites, which on reaching the electrode exerted a specific effect. Lactic acid suggested itself but the injection of sodium lactate did not produce the effects of massive injections of cyanide. The possibility of the liberation of more highly reducing substances might well be considered. Obviously cyanide experiments should be checked with other methods of pH determination. Unfortunately the quinhydrone electrode is also an oxidation-reduction electrode. Though Cullen and Billman demonstrated that it is possible to determine the initial potential due to the pH in serum our experiments on hemorrhage suggest that it will be a sound precaution to use the hydrogen electrode as the final check in experimental work in which reducing substances may vary as a result of disturbed metabolism. We hope to avoid misinterpretation of extraneous potential changes by observing this precaution and perhaps evaluate the significance of oxidation-reduction phenomena in respiratory control.

SUMMARY AND CONCLUSIONS

The need of a continuous method of recording rapid changes in acidity of the circulating blood and other body fluids for the study of respiratory control and acid-base equilibrium has led to the use of the manganese dioxide electrode.

By means of an electrode cannula the electrode is placed directly in the blood stream of a heparinized dog and connected in the usual way with a type K Leeds and Northrup potentiometer. The galvanometer is maintained in balance and the changes in E.M.F. mechanically recorded on
smoked paper by writing points connected with thread to the fine adjustment of the potentiometer.

Reproducibility of behavior of individual electrodes in dissimilar fluids and blood plasmas from different animals was not attained but a high degree of reproducibility of behavior to changes in acidity in the same sample was the rule. Four electrodes placed in the stream of alternating fluids of different pH values gave mutually superimposable curves.

By establishing the position of the curves with standard methods the manganese dioxide electrode would, therefore, seem to meet the requirements for many biological problems. A comparison of records of acidity changes in the circulating blood with curves established by the discontinuous method of pH determination on individual blood samples offered direct proof of the value of the method in animal experiments. On the administration of carbon dioxide, low oxygen, sodium carbonate, ammonium chloride, lactic acid, small amounts of sodium cyanide, in mechanical asphyxia, and in hemorrhage and injection there was close agreement in the behavior of the manganese dioxide electrode and the hydrogen and quinhydrone electrodes. The only gross exception so far discovered was the discrepancy in results with massive injection of sodium cyanide. It was suggested that this discrepancy was due to the increased liberation of reducing metabolites.

Precaution against the effects of these reducing substances was stressed.

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