

JAN 5 1965

DIST A54 0199

Transient Response of the Glass Electrode to pH Step Variations

A. DISTÈCHE AND M. DUBUISSON

Reprinted from *THE REVIEW OF SCIENTIFIC INSTRUMENTS*, Vol. 25, No. 9, pp. 869-875, September, 1954

Transient Response of the Glass Electrode to pH Step Variations

A. DISTÈCHE AND M. DUBUISSON
Laboratory of General Biology, University of Liège, Belgium
(Received November 18, 1953)

A dc amplifier with electrometer input stage is described making it possible to display pH changes on the oscilloscope screen for physiological applications. Sensitivity is 0.003 pH unit per cm beam deflection; noise 50 μ v; drift 300 μ v/hr (\approx 0.005 pH unit). A simple hydraulic mechanism is used to produce very fast pH steps under the electrode. The speed of the electrode response is shown to tend towards a finite limit when the speed of the pH change increases. The form of the transient in the fastest records is close to an exponential curve $e^{-\alpha t}$, with $1/\alpha \approx 30$ msec. The response is independent of the membrane thickness (5 to 10 μ). Some evidence exists to show that the response of the electrode to pH steps is analogous to the response of a low-pass RC-filter to a corresponding voltage step, where R and C are, respectively, the resistance and the capacity of the glass membrane. R and C values determined from the analysis of the electrode response to step voltages in series give $RC \approx 30$ msec in agreement with the experimental results.

INTRODUCTION

AS shown by Dubuisson,¹⁻⁷ pH changes which take place during muscle activity can be detected by means of a glass electrode with its flat active membrane in close contact with the muscle surface through a thin film of Ringer solution. A twin-tube balanced electrometer stage (Dubuisson and Debot)⁸ and a galvanometer were used for photographic recording. The time required for an instantaneous pH change to be recorded as a full galvanometer deflection was found experimentally to be 4 sec, corresponding to the galvanometer period. This rather high delay does not bring too much distortion in the recordings of the pH variations which occur during the contraction of a smooth muscle (Dubuisson),⁵ or during slow stretching of striated muscle (Dubuisson),⁶ but makes it impossible to study the pH changes during a single twitch of the striated muscle of a frog.

As pointed out at the time, besides the time lag due to the recording instrument, one has to know the delays introduced by the intrinsic properties of the electrode and by the diffusion processes which characterize the CO₂ exchanges between the muscle fibers and the film of Ringer under the glass membrane.

A theoretical estimation of the delay brought by diffusion has been given by Hill.⁹

As a first attempt to improve the technique, we have made the delay due to the recording device (electrode excluded) negligible, by feeding the electrometer stage output to a dc high gain amplifier in order to display the pH changes on the oscilloscope screen. This has

been possible thanks to the very great help received from A. V. Hill and his collaborators.

The next step was to measure the delay introduced by the electrode by recording its transient response to an instantaneous pH change.

I. EXPERIMENTAL TRANSIENTS CORRESPONDING TO VERY FAST pH STEP VARIATIONS

Technique

A. Electrometer, DC Amplifier, Oscilloscope

The electrometer circuit is shown in Fig. 1. It is essentially similar to that described by Dubuisson and Debot,⁸ except that the voltage gain is increased from $\times 0.3$ to $\times 0.7$ by using higher plate loads and a higher plate supply voltage. The circuit was initially designed for large-sized Gecovalve ET1 electrometer triodes, but as the Gecovalve ET3 sub-miniature triodes proved less microphonic, they were preferred.

The grid current is sufficiently small for potential measurements with glass electrodes of 100 megohms internal resistance: identical galvanometer deflections are obtained for a given dc input

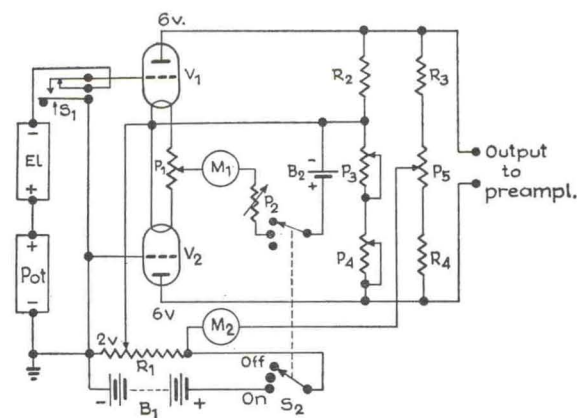


FIG. 1. Circuit diagram of valve electrometer for pH measurements. $R_1=12K$ with tap. $R_2=250K$. $R_3, R_4=35K$. $P_1=5\omega$ single wire pot. $P_2=25\omega$ single wire pot. $P_3=50K$ helical pot. $P_4=5\times 45K$ on S.p.-6 pos.-rotary sw. $P_5=10K$ helical pot. EL =electrode assembly (see page 870). $Pot.$ =precision potentiometer. (All resistors are precision wire wound.) S_1 =make-before-break high insulated switch. S_2 =D.p.-3 pos.-rotary sw. $M_1=0-50$ mA. $M_2=0-500\mu A$. $B_1=12$ -v battery. $B_2=2$ -v. battery. V_1, V_2 =Gecovalve ET3 sub-miniature electrometer triode.

¹ M. Dubuisson, Proc. Soc. Exptl. Biol. Med. **35**, 609 (1937).
² M. Dubuisson, Proc. Physiol. Soc. Phila. **90**, 1 (1937).
³ M. Dubuisson, Pflügers Arch. ges Physiol. **239**, 314 (1937).
⁴ M. Dubuisson and W. Schulz, Pflügers Arch. ges Physiol. **239**, 776 (1938).
⁵ M. Dubuisson, J. Physiol. (London) **94**, 461 (1939).
⁶ M. Dubuisson, Arch. intern. physiol. **50**, 203 (1940).
⁷ M. Dubuisson, Proc. Roy. Soc. (London) series B, **137**, 63 (1950).
⁸ M. Dubuisson and A. Debot, Arch. intern. physiol. **50**, 54 (1940).
⁹ A. V. Hill, Proc. Roy. Soc. (London) series B, **135**, 446 (1948).

potential whether fed to the grid through a 100-megohm resistor or directly.

As this instrument is intended to record continuous pH changes to within 0.01 pH unit and less, great care has to be taken to eliminate the zero drift. It can be shown experimentally that with carefully matched tubes, there is always a setting of potentiometer P_1 (heater current balance), for which one is able to find a setting of potentiometer P_3 (plate load balance) which suppresses the zero drift between plates, or reduces it to a very small value. Once this equilibrium is reached, a slight change to the right or to the left in the setting of potentiometer P_3 starts the drift in one or the other direction. The equilibration is carried out with a sensitive galvanometer with suitable shunt, plugged in between plates. To shift the zero P_3 and P_4 are used. At minimum drift point, provided the total filament current is always adjusted to the same value and the filament supply battery is maintained in the flat portion of its discharge curve, the H.T. battery can be used down $\frac{2}{3}$ of its discharge curve without drift trouble. The minimum drift reached this way is always continuous. Spurious drifts and jumps indicate defective batteries, poor connections, insufficient insulation (input grid circuit, grid switch, electrode assembly, moisture, or dirt on the electrometer valves), sudden temperature changes, etc. The usual precautions against such sources of disturbance have been taken.

The major trouble when the output of the ET1 and ET3 electrometer valves is amplified and displayed on the oscilloscope screen, the amplification factor being as high as $\times 190\,000$, is their very marked microphony. To eliminate vibrations and sound pick-up, the valves have to be mounted in an electrically shielded sound-proof cabinet, on a spring suspension of the type designed by Hill¹⁰ for vibrationless galvanometer mountings, and similar to the system described by Frankenhaeuser and Mac Donald.¹¹ The chassis with the circuit parts and controls, including the highly insulated grid switch, is fixed externally on the cabinet, which is firmly secured to the room-wall. The grid lead from the electrode assembly is a rigid high-insulated shielded cable of the coaxial

type, and care is taken to avoid having vibrations transmitted to this cable.

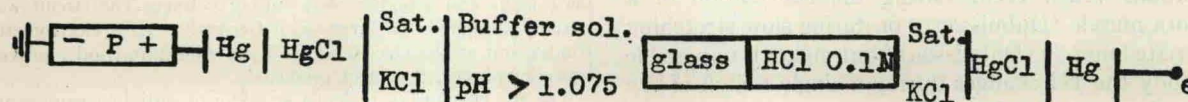
The electrometer stage is followed by a battery operated dc preamplifier. Its gain is $\times 300$, and it is similar to that described by Copeland.¹² It has four valves: two type E37A, operating as a long tailed-pair whose outputs are converted to a single-sided signal by a 6SC7 valve and fed at earth level to the oscilloscope amplifier by a cathode follower (6J5). The oscilloscope dc amplifier is the high gain channel ($\times 900$) of the Cossor model 1049 double-beam oscilloscope with camera attachment.

The gain of the electrometer stage being $\times 0.7$, the over-all full voltage gain of the whole instrument is $\times 190\,000$. The tube sensitivity is $160\ \mu\text{v}/\text{cm}$ ($\approx 0.003\ pH/\text{cm}$). The oscilloscope attenuator is used for different gain settings. The signal acceptance at full gain without distortion is 50 mv. The over-all drift, after a 1 hr warmup, at full amplification, is about $300\ \mu\text{v}/\text{hr}$ ($\approx 0.005\ pH$ unit). The drift is continuous, without sudden jumps. The noise level is about $50\ \mu\text{v}$ peak to peak (band width reduced to dc-4.6 kc/sec, input grid earthed or connected to the electrode assembly). Stepwise reduction of the band width is provided for, down to dc-50 c/sec, the peak to peak noise decreasing to less than $20\ \mu\text{v}$.

B. Glass Electrodes, Calomel Electrodes, and Cell Assembly

The glass electrodes are of the Mac Innes and Dole type,¹³ and made by sealing a thin membrane of 015 Corning glass onto an ordinary glass tube (inner ϕ 6.5 mm, outer ϕ 8 mm). The extremity of the glass tubes is ground flat and perpendicular to the stem axis. The tubes are 10 or 12 cm long, and coated with paraffin wax up to 2 or 3 cm from the membrane. Electrodes with skew, rippled, or sucked-in membranes are discarded.

The inside of the electrode is filled with 0.1 N HCl, and the electrode is connected in the following cell assembly:



(P is the compensating potentiometer; e is connected to the input grid of the electrometer.)

The calomel electrodes are of the conventional type and care is taken to connect them in opposition during 48 hrs after filling with saturated KCl, to eliminate any potential difference. The salt bridges are filled with agar-agar jelly saturated with KCl.

The glass electrodes are checked by measuring their potential in an acetate buffer pH 4.66. The electrodes giving stable potentials between 205 and 215 mv are retained.

The membrane thickness of the electrodes ranges from 5 to $10\ \mu$. These values have been found by comparing the edge thickness of about 100 glass flakes taken from the bulbs blown to make electrodes, to a calibrated wire gauge under a microscope with micrometer scale.

C. Equipment to Produce a Step pH Change Under the Electrodes

To measure the distortion introduced by the glass electrode in the recording of fast pH changes, the shape of the pH step variation has to be known. As the response of other electrodes, sensitive to H^+ ions or other ions, is unknown,* there is no direct

way to solve this problem. An indirect way is to produce a pH change so fast compared to the delay due to the electrode, that it can safely be assumed to approximate a rectangular step.

Fast pH changes can be produced mechanically, either by mixing solutions of different pH under the electrode, or by quickly displacing one solution by another. The shape of the pH variation will depend on the mechanical arrangement and may be affected by diffusion, adsorption, viscosity, and surface wetting. The faster the pH variation, the less its shape will depend on the first two of these factors, the last three appearing as speed limiting factors for a given solution.

Mixing was first tried, but soon proved to give irregular and too-slow pH changes, however vigorous the stirring.

The quick displacement of one solution by another of different pH gave much better results from the start. After much trial, the apparatus shown in Fig. 2 was built.

The inverted glass bottle A , the rubber tube B , the Y-shaped glass tube y , are filled with solution 1. The liquid does not flow as long as valve a is closed. A droplet of solution 2 ($pH_1 - pH_2 = 0.3$) is suspended under the electrode E . When valve a is opened, solution 1 bursts out of the flattened tip of tube y and washes

depends very much on the degree of polish of the metallic surface. With the specimens tried, the response has been found much slower than that of a glass electrode. Only a few experiments have been made, and no conclusions can be safely drawn.

¹⁰ A. V. Hill, *J. Sci. Instr.* **25**, 225 (1948).

¹¹ B. Frankenhaeuser and D. K. C. Mac Donald, *J. Sci. Instr.* **26**, 145 (1949).

* Some experiments have been tried with a platinum electrode and quinhydrone, and with a Cu/CuSO₄ electrode of the same shape as the glass-electrode. The response of these electrodes

¹² K. Copeland, *J. of Physiol. (London)* **117**, 15 P (1952).

¹³ D. A. Mac Innes and M. Dole, *J. Am. Chem. Soc.* **52**, 29 (1930).

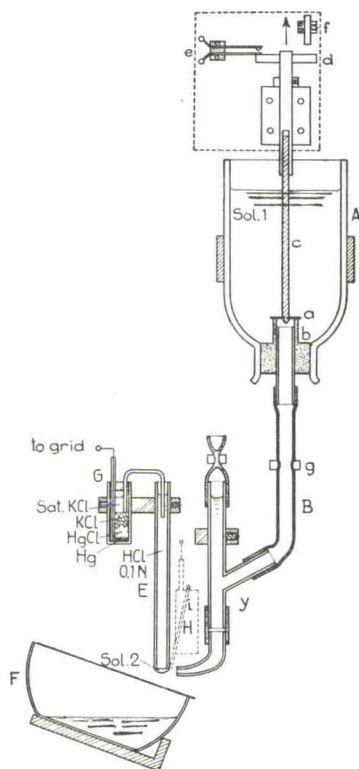


FIG. 2. Schematic diagram of an apparatus to produce pH step variations under a glass electrode (see text).

solution 2 away. The liquid is collected in glass vessel *F*. The whole apparatus is carefully insulated from ground and shielded. Valve *a* is simply a soft rubber washer fitted in shaft *c* and held tight by hydrostatic pressure on the rim of a rigid rubber tube *b*. The valve is operated manually and opens by pushing button *d* (on top of shaft *c*) upwards to stop *f*. This movement simultaneously closes electrical switch *e* which starts the time base of the oscilloscope. The under part of shaft *c*, in the solution, is ebonite, the upper part is brass and earthed (parts *d*, *e*, *f*, and the guide through which *c* slides are fixed on a wooden block, represented by a dotted line, behind the plane of Fig. 2).

The flow is regulated by means of clamp *g*. Tube *y* is fitted with a removable flattened tip to obtain a ribbon-like jet. Its vertical branch serves to trap air bubbles which may appear at the tip when the flow is interrupted. Any orientation of tube *y* is possible. To fill the apparatus, the tube is removed from its clamp and leveled with bottle *A*, which is 50 cm above. The air bubble trap is filled up to the top.

The glass electrode *E*, and its top calomel electrode *G*, are fixed in a highly insulated ebonite clamp which can be oriented in any position. The whole electrode assembly can be moved smoothly along its vertical axis by screw action. The second calomel electrode *H* (in dotted line) is fixed to a separate insulated stand and is located behind tube *y* and electrode *E*. Contact with the droplet of solution 2 under the electrode is made through a thin agar-agar filled bridge *i* with a few millimeters of cotton thread sticking out of the tip. The bridge is fixed so as not to be blown away by the liquid jet, and only the cotton thread is in contact with the liquid.

The main difficulty with this apparatus is in aligning the electrode and jet so that proper grazing of the suspended droplet is obtained. To make this easier, experiments have been made with a slightly modified arrangement. A thin rectangular flat-bottomed celluloid channel is fitted horizontally under the tip of tube *y*. The drop of solution which has to be washed away is squeezed

between the electrode membrane and the dried celluloid plate, forming a liquid layer 1 or 2 mm thick, located at 2 mm from the tip end.

Results

It soon became evident, after the first experiments, that the response of the glass electrode is so fast that it is very difficult to produce, mechanically, a pH change fast enough to approximate a rectangular step compared to the transient response of the electrode. The efficiency of the washing depends very much on the respective orientation of jet and electrode. The difficulty with the described apparatus is to direct the jet properly on the electrode to wash the droplet away as quickly and completely as possible. When the jet breaks on the electrode stem, there is much turbulence, the liquid creeps upwards, mixing occurs under the membrane before the droplet is removed. When the jet reaches only the droplet, part of it remains under the electrode. Diffusion complicates the process still further in both cases. Parallelism between membrane and jet, form and section of the injection tip, and flatness of the electrode membrane, are all factors of importance.

With the celluloid channel arrangement, alignment is more easy and reproducible, but much slower variations are obtained. The droplet squeezed between two planes is more difficult to remove. Much liquid flows around the electrode and produces turbulence of flow, which may result in stationary mixing under the electrode, as was discovered with dyed solutions.

For a given spatial arrangement the washing efficiency passes through a maximum range when the flow is increased. Higher flow rates are possible with the free-hanging drop method than with the channel method, before too much turbulence makes it illusory to increase the flow still further.

TABLE I. Milliseconds at percent total deflection.^a

Experiment <i>n</i> ^o	20%	40%	60%	80%	90%	100%
<i>A</i> ₈ 1	23	44	61	87	122	192
2	21	31	44	65	87	200
<i>A</i> ₁₇	21	38	61	105	166	350
<i>A</i> ₁₈	21	38	61	80	105	228
<i>A</i> ₁₉	21	35	58	87	114	280
<i>A</i> ₂₇ 1	31	39	56	75	96	140
2	30	39	52	79	96	140
<i>A</i> ₄₀ 1x	24	38	54	78	102	180
2x	20	36	52	72	92	180
3x	20	32	50	68	88	160
<i>A</i> ₄₈ 2	16	25	39	52	70	175
9	18	25	44	87	122	350
<i>A</i> ₄₉ 1x	12	20	28	44	64	160
2	18	26	37	44	70	260
7x	12	22	28	40	60	100
9x	18	22	32	45	68	140
10x	12	20	28	44	68	130
<i>A</i> ₆₀ 8	18	26	39	52	122	260
9	18	26	39	61	140	350

^a Remarks: *A*₈–*A*₂₇ phosphate buffer; *A*₄₀–*A*₆₀ acetate buffer; *A*₈–*A*₄₀ channel method of injection; *A*₄₀, flow rate increased from 1 to 3; *A*₄₈–*A*₆₀ free hanging drop method of injection; *A*₄₉ different flow rates; x, values measured on enlarged records, the other values are measured directly on the film.

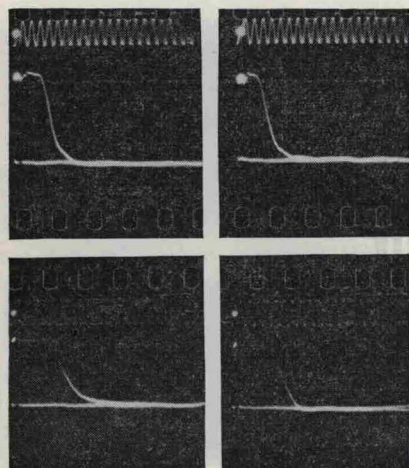


FIG. 3. Oscillographic records of fast pH step variations under a glass electrode (experiments $n^{\circ}A_{40}$, below, and A_{49} , above, from Table I). Time-scale: 50 c/sec sine wave.

The high dc resistance of the electrode also complicates the problem. Electrostatic disturbances and mechanical vibrations, due to the jet impact, are picked up and appear as spurious jumps, overshoot, and oscillations in the amplifier output. These effects become very large at high jet speeds, especially when the liquid reaches the paraffin wax coating of the electrode. At lower speeds, a slight overshoot or, sometimes, a potential drop at the beginning of the transient can still be detected in almost any experiments with free-hanging droplets, but is much less frequent when a channel is used. An explanation is that the insulated injection apparatus may have a different electrostatic charge than the glass electrode, whose tip

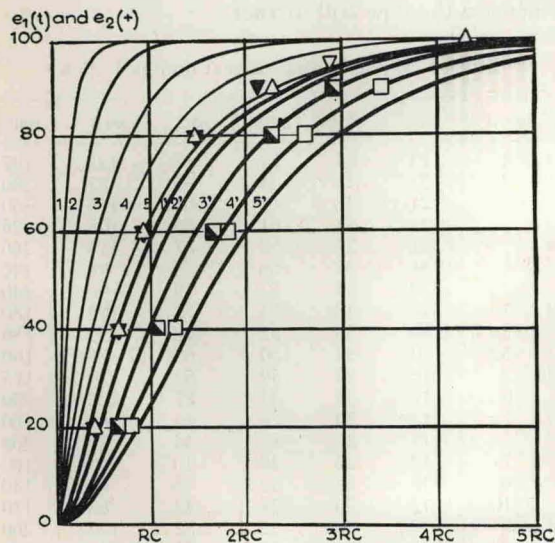


FIG. 4. Calculated responses (smooth curves 1' to 5') of a glass electrode to exponential pH step variations of type $e^{-\beta t}$ for different values of β (smooth curves 1 to 5). Curve 5 represents also the response of the electrode to a rectangular pH step. The plotted data are taken from Table I ($RC=30$ msec).

is earthed through the calomel electrode. In the free-hanging drop method, electrode and injection apparatus are separated by an air gap, whereas the celluloid channel provides a leakage path. If the conductivity of the more or less dried celluloid surface is increased by means of a wet cotton thread, fixed inside the injection tip and stretched across the channel so as to touch the bridge tip of the calomel electrode and to dip in the collecting vessel (see Fig. 2), the effects due to initial electrostatic charge difference between electrode and injection apparatus disappear almost completely. The thicker the membrane the more difficult it is to avoid electrical disturbances. As it soon appeared that *the membrane thickness has little or no effect on the response speed*, thin electrodes were prepared for most of the experiments.

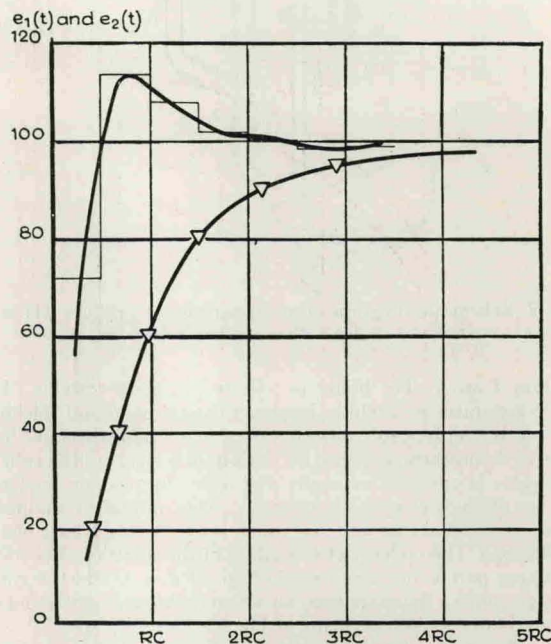


FIG. 5. Calculated pH variation (blocs) from the recorded response of a glass electrode (experiment $n^{\circ}49_1$ of Table I).

Perfect cleanliness and wettability of the electrode membrane are, of course essential to obtain quick responses, independently of the speed of the pH change. Unclean membranes slow the response considerably. Washing with organic solvents (to remove traces of paraffin wax, used to increase the resistivity of the glass stem), with concentrated $HNO_3 + H_2SO_4$, improves the response of unclean electrodes. It is difficult to keep the electrodes clean, even in distilled water, and we have used the electrodes as soon as they were made, after a few minutes soaking in the buffer solution. Better results are obtained with acetate buffer (0.1 N acetic acid + 0.1 N NaOH, pH 4.66) than with phosphate buffers (pH 7), because the latter prepared from crystallized Na and K salts, always contains grease which deposits on the membrane.

We will not look in detail at the experimental evidence of the difficulties described in the foregoing, and we will now consider some of the fastest transients with smooth and regular shapes which we have obtained and which are given in numerical form in Table I. Figure 3 shows the recorded curves of the best experiments. The experiments of Table I have been chosen to give the same arithmetical mean value for the time needed to reach 60 percent deflection, as found for the entire set of the 50 well-shaped curves which we have recorded.

The most likely interpretation of these results is that the response of a glass electrode to a rectangular pH step is an exponential curve of type $e^{-\alpha t}$ with a time constant $1/\alpha$ of about 30 msec. As we know nothing about the shape of the experimental pH variations no

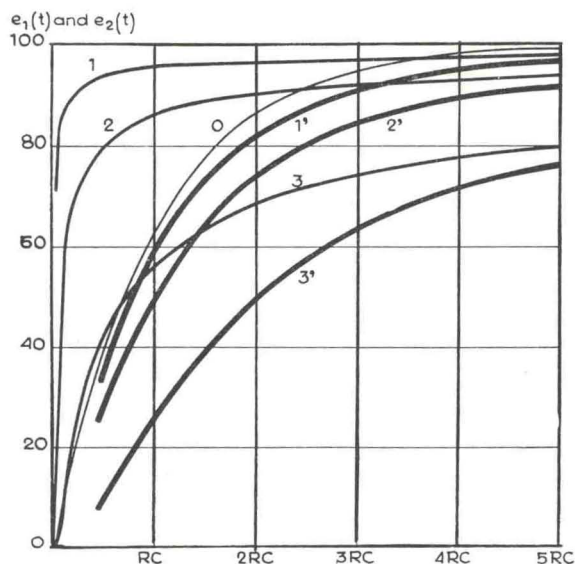


FIG. 6. Calculated response (curves 1', 2', 3') of a glass electrode to a rectangular pH step transmitted to the electrode membrane by diffusion through a thin film of liquid of 0.3μ (curve 1), 1μ (curve 2), 3μ (curve 3). The diffusion curves are drawn after Hill (see reference 9). Curve 0 is the electrode response to a rectangular pH step undelayed by diffusion.

absolute proof of this can be drawn from the data, but some arguments can be advanced.

Above a certain value of flow rate, important variations of jet speed with obvious improvement of the washing efficiency have but little effect on the response: in over 200 experiments 60 percent deflections are attained in 60 msec to 28 msec (10 experiments give values ranging from 140 to 60 msec).

Figure 4 shows the theoretical electrode response to exponential pH variations of type $e^{-\beta t}$ of increasing speed, assuming that the electrode response to a rectangular pH step is represented by $e^{-\alpha t}$ with $1/\alpha = 30$ msec. Some experimental data from Table I have been plotted on the diagram. It is seen that $1/\beta$ decreases from 23 msec to 4-7 msec, whereas the time at 60 percent deflection decreases from 54 msec to 28 msec, when

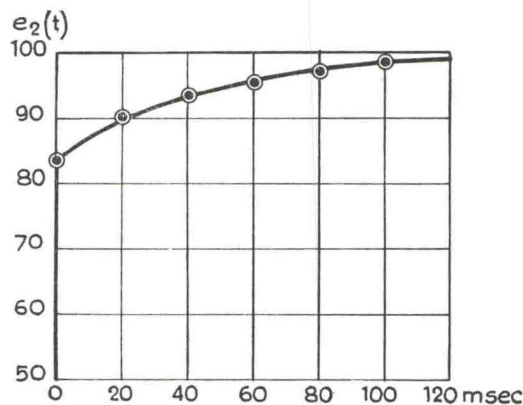


FIG. 7. Calculated (smooth curve) and experimental (0) response to a rectangular step voltage of the network of Fig. 9 with $r = 9.10^7$ ohms, $R = 10^8$ ohms, $C_1 = 400$ cm, $C_2 = 80$ cm.

the injection apparatus is used respectively with the channel method and the free-hanging drop arrangement. The value of 4-7 msec for $1/\beta$ is in agreement with the expected performance of the injection apparatus. The rate of the flow in the middle of the range used has been measured and found to be $20 \text{ cm}^3/\text{sec}$. The volume of the droplet under the electrode is $\sim 0.05 \text{ cm}^3$. It can be calculated that in ideal conditions (cross section of the drop inscribed in the rectangular section of the jet), the droplet could be removed in 6 msec.

The important curvature at the start of the slower transients, the much increased steepness in the fast ones, and the fact that the deviations from an ideal exponential curve decrease progressively as the speed increases, show the approach towards a limit as exemplified in Fig. 4. It will be noticed however that the plotted data corresponding to the fastest experiments reveal a slight deviation from the calculated curves. Analysis by the graphical and numerical method of Hill¹⁴ is given in Fig. 5, revealing an overshoot of 14 percent. An explanation for this phenomenon has been suggested earlier. The argument concerning the shape of the limit

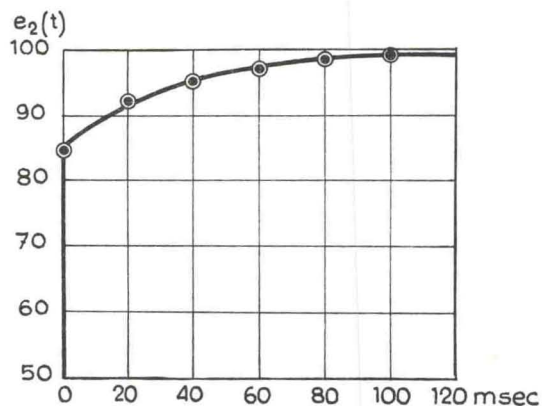


FIG. 8. Calculated (smooth curve) and experimental (0) response of a glass electrode ($n^\circ 95a$, see text) to a rectangular step voltage in series with the electrode.

¹⁴ A. V. Hill, J. Sci. Instr. 26, 56 (1949).

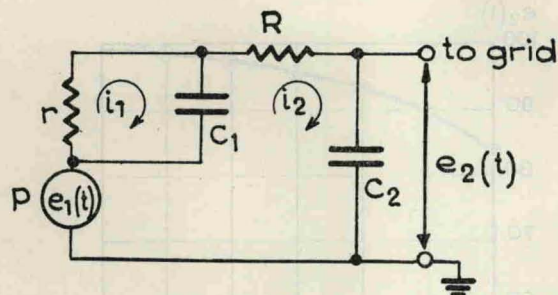


FIG. 9. Equivalent network for glass electrode and input connections to the electrometer, for responses to step voltages in series with the electrode.

transient is somewhat reduced by the fact that if the curves present great similitudes up to 80–90 percent deflection, important differences are observed in the last part of the deflection. The time required for full deflection ranges from 100 to 450 msec. The delaying factor is most likely diffusion, but we have no experimental evidence. If part of the pH variation is transmitted to the membrane by diffusion either through a film of liquid remaining on the membrane because of insufficient washing, or through a film of adsorbed dirt or impurities from the buffer solutions, the delay will be especially noticeable in the last 20 percent of the deflections because of the peculiar shape of the diffusion curves. This is shown in Fig. 6, which gives the theoretical transient response of the glass electrode to a rectangular pH step transmitted to the membrane by diffusion through a thin film of liquid. The response curves are calculated by numerical and graphical analysis,¹⁴ again assuming $e^{-\alpha t}$ to represent the electrode response with $1/\alpha = 30$ msec, when there is no diffusion. The diffusion curves are taken from Hill⁹ (diffusion constant $= 5 \cdot 10^{-6}$). It is obvious that the electrode response is severely slowed by diffusion even with films of less than 0.3μ , and comparison with Fig. 4 shows clearly that the delay is especially marked in the last 20 percent of the response curve.

II. ELECTRICAL CHARACTERISTICS OF THE GLASS ELECTRODE

Sending a step-voltage of a few millivolts through the electrode assembly (by closing a key across the terminals of an auxiliary potentiometer in series with compensating potentiometer of Fig. 1) gives a response curve like the one shown in Fig. 8. Figure 7 shows a similar curve obtained with the circuit¹⁵ of Fig. 9, with $r = 90 \cdot 10^6$ ohm, $R = 10^5$ ohm, $C_1 = 400$ cm, $C_2 = 80$ cm (input capacity of electrometer). Both curves correspond to the equation:

$$e_2(t) = V \left[1 - \frac{C_2}{(C_1 + C_2)} e^{-t/r(C_1 + C_2)} - \frac{C_1}{(C_1 + C_2)} e^{-t((C_1 + C_2)/C_1 C_2 R)} \right]. \quad (1)$$

¹⁵ A similar equivalent circuit for the glass electrode has been suggested by D. A. Mac Innes and D. Belcher, *J. Am. Chem. Soc.* **53**, 3315 (1931).

In the case of the glass electrode, C_2 and R (calomel electrodes + salt bridges + potentiometer $= 30\,000$ ohm) being known, C_1 and r (capacity and internal resistance of the glass membrane) can be calculated as follows:

Electrode n°	C_1 cm	$r \cdot 10^6$ ohm	Eq. (1)
95a	460	65	$e_2(t) = V(1 - 0.15e^{-28t} - 0.85e^{-5 \cdot 10^6 t})$
95b	600	45	$e_2(t) = V(1 - 0.12e^{-33t} - 0.88e^{-47 \cdot 10^6 t})$

Direct measurement of C_1 and r with capacitor and megohmmeter gives the following values: 95a: $C_1 = 460$ cm, $r = 300 \cdot 10^6$ ohm, $rC_1 = 138$ msec; 95b: $C_1 = 600$ cm, $r = 240 \cdot 10^6$ ohm, $rC_1 = 133$ msec; other electrodes give respectively: $C_1 = 460, 380, 610, 510$ cm $- r = 300, 350, 240, 290 \cdot 10^6$ ohm $- rC_1 = 146, 148, 138, 144$ msec.

The discrepancy between the calculated and measured values of r is difficult to explain, but may be the result of polarization in the latter case.¹⁵

In Fig. 10, we have rearranged the circuit of Fig. 9 to represent the electrode transmitting a pH step, the electrode being taken as an emf source, without taking into account the internal mechanism for the conversion of the pH change into the corresponding step voltage. The response of this circuit where $r \gg R$, $C_1 > C_2$ is given by equation:

$$e_2(t) = V[1 - e^{-t/r(C_1 + C_2)}]. \quad (2)$$

Using for r and C_1 the values calculated from Eq. (1), the recorded time constant of the electrode $r(C_1 + C_2)$ can be expected to be 36 msec and 30 msec for electrodes 95a and 95b, the true time constant rC_1 being, respectively, 30 and 27 msec. This picture of the electrode transmitting the voltage corresponding to a pH step as a low-pass RC circuit fits well with our experimental conclusions, e.g., 1° the speed of the electrode tends towards a finite limit as the speed of the pH change increases; 2° the form of the transient of the fastest records is very near to an exponential curve of type $e^{-\alpha t}$ with $1/\alpha \approx 30$ msec; 3° the response is independent of the electrode thickness.

REMARKS. (1) If the band width of the electrode transmitting pH oscillations (sine wave) is 5 c/sec, it is interesting to notice that pH oscillations at 14 c/sec and 50 c/sec will be attenuated to $\frac{1}{3}$ and $\frac{1}{5}$ of their real amplitude. Discrimination between pH step

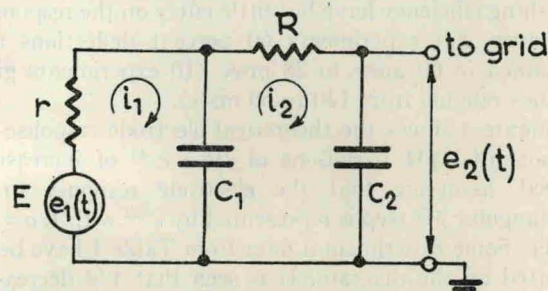


FIG. 10. Equivalent network for glass electrode and input connections to the electrometer, for responses to pH step variations.

variations reaching 60 percent deflection in less than $\frac{1}{8}$ th the electrode time constant (~ 4 msec) will be impossible (see Fig. 4).

(2) Electrical signals appearing between any two points of the resistor formed by the liquid under the glass electrode, the junction electrode and the potentiometer will be picked up by the electrode (electrical stimuli applied to biological preparation, action potentials, etc.). The electrode in this case (Fig. 9) forms a kind of high-pass filter with a band width value as high as 165 kc/sec if silver chloride electrodes of 3000 ohms are used. It follows that discrimination between oscillating electrical signals and pH

changes in the output records will be easy above 50 c/sec for the former.

(3) Negative feedback from the preamplifier output to electrometer input grid increases the band width of the electrode for electrical signals (the electrode time constant is easily lowered to about 2 msec). The effect of feedback on the response to pH changes has not been studied: the gain loss is too great and the apparatus becomes unsuitable for physiological work. This question will be taken up again if some additional gain can be obtained from a modified version of the preamplifier.