two possible actions appear indistinguishable on the basis of evidence obtained from short-term leukocyte cultures, the technique apparently has value in the selection of potential carcinogens for long-term testing programs.

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2. Medium 199 was supplemented with 20 percent calf serum (Grand Island Biological Co.) and 2.5 percent (by volume) phytohemagglutinin M (Difco Laboratories). Blood was obtained by venipuncture from a 25-year-old male with no recent viral infection, medication, or diagnostic irradiation.
3. Gaps and breaks are defined as achromatic lesions without and with displacement of the fragment, respectively.
4. Mode 1 percent; range 0 to 3 percent per culture.
5. We thank Drs. A. B. Morrison, W. P. McKinley, and H. C. Grice for advice and encouragement.

20 November 1969; revised 5 January 1970

Unified Account of the Variable Effects of Carbon Dioxide on Nerve Cells

Abstract. *When the abdominal ganglion of* Aplysia californica *is exposed to 5 percent carbon dioxide, certain neurons are depolarized, others hyperpolarized, and some are unaffected. The effect of increased carbon dioxide is due solely to the concomitant fall in extracellular* p*H, which causes an increase in membrane chloride conductance of responsive cells. The directional change of the membrane potential in different neurons is determined by the relative values of the chloride equilibrium and the resting potentials. The chloride equilibrium potentials are calculated after direct measurement of the intracellular chloride activity with a chloride microelectrode.*

Increased CO_2 has different effects on different nerve cells. For example, it causes hyperpolarization and depression of cortical neurons (*1*) and of phrenic (*2*) and lumbar motoneurons (*3*). On the other hand, it causes depolarization and excitation of neurons of *Aplysia* and *Helix* (*4*) and of respiratory center neurons of mammals (*5*). In the carotid sinus of mammals chemoreceptors are excited whereas baroreceptors are unaffected (*6*). The purpose of our experiments was to determine the precise mechanism by which CO_2 acts, and to account for the differences in response among different nerve cells. The results show that the effects of CO_2 are due entirely to the concomitant decrease in extracellular *p*H, which produces an increase in membrane chloride conductance. The ensuing directional change of membrane potential and hence neuronal excitation or depression is determined by the relative values of chloride equilibrium potentials and membrane resting potentials.

The abdominal ganglion of *Aplysia californica* was removed and superfused with artificial seawater (ASW), *p*H 8.0, having the same composition as extracellular fluid (*7*). The buffer was 10 m*M* tris maleate, and the buffer curve of ASW had a constant slope over a *p*H range 3 to 10. A CO_2–bicarbonate buffer system was used occasionally without affecting the results. Two to three cells at a time were impaled with glass micropipettes; conventional recording and stimulating techniques were followed (*8*). Intracellular chloride activities were measured with a chloride microelectrode constructed as follows.

A vertical pipette puller (Kopf model 700B) is used to pull a micropipette from a piece of Pyrex (Corning code 7740) capillary tubing that has been cleaned with hot ethanol vapor. Immediately after being removed from the puller the pipette is dipped for 5 to 10 seconds in a silicone solution of 2 percent by volume of Siliclad in 1-chloronaphthalene. After being dipped the pipette is examined under × 100 magnification to be sure that there is a column of the solution 75 to 100 μm long inside the tip, and the pipette is then placed tip up in a drill hole in a metal block. As soon as the desired number of pipettes have been pulled and dipped they are put into a 250°C oven for 1 hour. Upon removal from the oven the pipettes are covered and left standing tip-up until they are to be used. Pipettes can be stored in this condition for at least 2 weeks.

One to two hours before being used the silicone-treated pipette tip is dipped in liquid chloride ion exchanger (Corning 476131) for 30 to 45 seconds. This puts a column, 100 to 150 μm long, of the organic liquid inside the tip of the pipette while the outside remains free of the ion exchanger. The remainder of

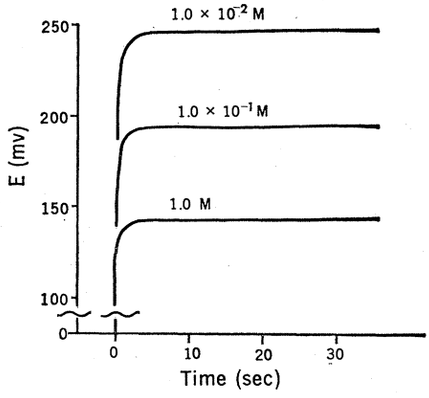


Fig. 1. Electrode potential plotted against time to show the time response of a typical electrode when it was dipped into KCl solutions of the concentrations shown in the figure.

the pipette is filled as far as possible with aqueous 1*M* KCl by means of a No. 30 needle and syringe; then a fine glass needle mounted on a micromanipulator (Narashige model MM3), while being viewed with × 100 magnification, is advanced down the inside of the pipette until it touches the liquid ion exchanger. Thereupon the pipette fills by capillarity down to the surface of the ion exchanger. If there are any air bubbles they can easily be removed with a cat whisker or a fine metal wire, manipulated by hand. The filled pipette is then stored with tip down in 1*M* KCl until it is to be used.

All electric potential measurements were made with the ion specific electrode connected to the input of a vibrating reed electrometer (Cary model 401) and with the reference electrode (calomel or Ag–AgCl with a saturated KCl bridge) connected to ground. The electrometer output was displayed on a digital voltmeter (Fairchild model 7050).

Electrodes, prepared as described above, have an anion slope of 56 ± 1 mv per tenfold change in activity at 25°C, independent of the cation in hydrogen, sodium, and potassium chloride solutions, over the concentration range of 1.0×10^{-3} to 1.0*M*. The response of the electrode to two other anions, bicarbonate and isethionate, was examined because they may be present in appreciable quantities in intracellular fluid (*9*). Equation 1 was used to calculate the response of the electrode to these anions relative to its response to chloride ion (*10*).

$$E = E_0 - \frac{nRT}{F} \log_{10} (a_{Cl} + K_i a_i) \quad (1)$$

E is electric potential (volts); E_0 is a constant (volts); R, the gas constant (8.2 joule deg^{-1} mole^{-1}); T, the temperature (°K); F, the Faraday (96,500 coulomb per equivalent); n, an empirical constant chosen so that nRT/F is the slope of the line when E is plotted as a function of $\log_{10} a_{Cl}$ in the absence of competing anions; a_{Cl} is the chloride ion activity; a_i is the activity of the competing anion; and K_i is the selectivity constant for the ith anion with respect to chloride. For bicarbonate and isethionate K_i was found to be 0.05 and 0.2, respectively, in mixtures with a constant concentration of 1.0M.

The chloride activities in the mixtures were measured with a Ag–AgCl electrode; a calomel electrode with a saturated KCl bridge was used as the reference electrode. The activity of the competing anion in pure 1.0M solution was taken as being equal to the cation activity as measured with a cation specific glass electrode against a calomel–KCl reference electrode. The activity coefficients of the competing anions in the mixtures were then calculated by the method of Guggenheim (*11*). Although this method for determining activity coefficients is not highly accurate, it is more reliable than a theoretical calculation using an extended Debye-Hückel equation and assuming values for the unknown parameters therein.

Figure 1 shows the time response of one electrode when it is dipped into a KCl solution whose concentration is either ten times greater or ten times less than that of the previous solution. The curves are labeled with their respective KCl concentrations.

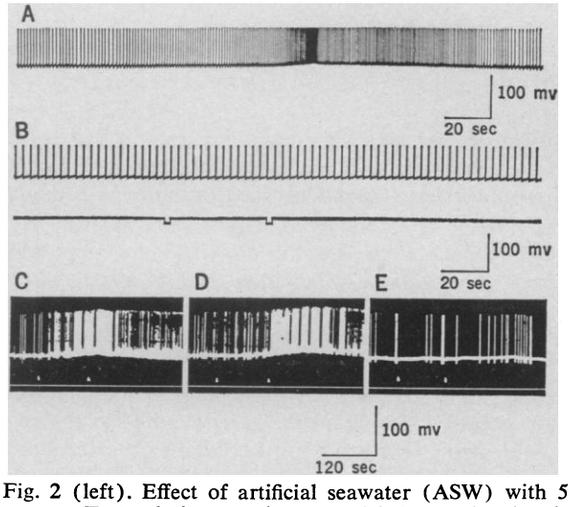
The resistance of the electrodes, which would be approximately 10^7 ohms if they were filled with 3M KCl, was calculated from the time constant to be 10^{10} to 10^{11} ohms. The time constant was estimated to be 0.5 to 1.0 second by allowing the electrode to come to equilibrium in a solution, discharging the capacitor of the electrometer, and then measuring the time course of reestablishment of the equilibrium potential.

These electrodes are stable to ±0.5 mv, with no change in slope, during the course of as many as eight cell penetrations over a period up to 3 hours. A discussion of liquid ion-exchange microelectrodes has been presented by Walker (*12*).

Figure 2 shows the response of A, a visceromotor cell, and B, a pacemaker cell, to ASW with 5 percent CO_2, pH 6.5. Visceromotor cells (*13*) and half of the giant cells showed an increased rate of discharge due to membrane depolarization; the rest of the giant cells were hyperpolarized. Pacemaker cells were little affected by CO_2 or decreased pH. Responses occurred within 30 seconds and were maximal between 90 and 240 seconds, after which the control solution was reintroduced. Recovery of resting potential and membrane resistance was complete after 15 to 60 minutes. In responsive cells, the identical effect to that evoked by ASW with 5 percent CO_2, pH 6.5, was elicited by ASW with no CO_2, pH 6.5, the pH being adjusted with HCl or H_2SO_4 (Fig. 2, C and D). However, ASW with either 5 or 50 percent CO_2, with pH held at 8.0 by continual titration with NaOH, had no effect, despite the fact that intracellular pH had almost certainly decreased [Fig. 2E; (*14*)].

The giant cell was either depolarized or hyperpolarized by CO_2 and decreased pH. In either event the membrane resistance fell by about 40 to 50 percent (Fig. 3, A and B). As shown in Fig. 3, C and D, the slope of the line relating membrane potential to external chloride ion concentration was doubled by a fall in pH from 8.0 to 5.0, indicating that the chloride conductance was doubled (*15*). The potassium conductance, however, was reduced by 25 percent. These changes in conductance accounted satisfactorily for the changes in membrane resistance.

Intracellular chloride activity, a^i_{Cl}, was measured in 14 giant cells. In seven cells, this value was 27.7 ± 1.8 mM (S.E.M.) and ranged from 21 to 31 mM. The chloride equilibrium potentials, E_{Cl}, calculated with the Nernst equation, were −63.6 ± 1.7 mv and ranged from −57 to −70 mv. For these cells, the resting membrane potential (E_m) was −56.4 ± 1.1 mv, and, in two



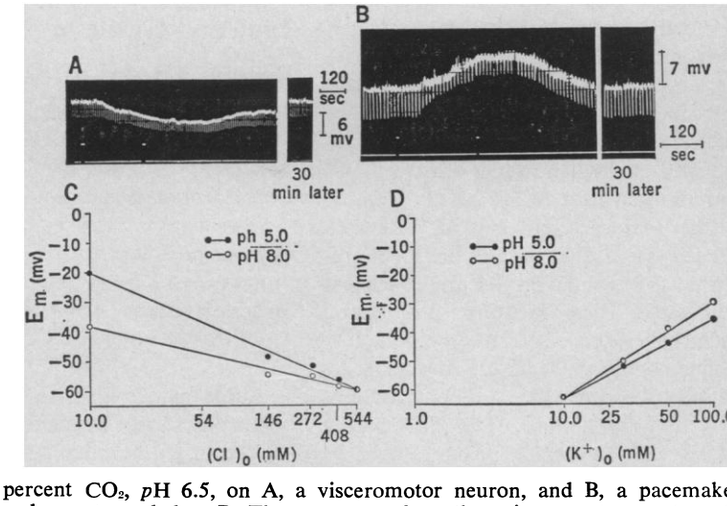


Fig. 2 (left). Effect of artificial seawater (ASW) with 5 percent CO_2, pH 6.5, on A, a visceromotor neuron, and B, a pacemaker neuron. Test solution was introduced between the signal marks on trace below B. The response of another visceromotor neutron to ASW with 5 percent CO_2, pH 6.5 (C), ASW with no CO_2, pH 6.5 (D), and ASW with 5 percent CO_2, pH 8.0 (E), is shown below. Test solutions were added between the signal marks on the bottom trace of each panel. Responses in C and D are almost identical; test solution in E had no effect. Fig. 3 (right). Effect of lowering pH from 8.0 to 5.0 on membrane potential, membrane resistance, and chloride and potassium conductances of the giant cell. Downward deflections in A and B are voltage deflections produced by constant current pulses (10^{-8} amp), 1.5 seconds in duation, every 10 seconds. Between signal marks (bottom trace), pH was decreased from 8.0 to 5.0. The giant cell in A was hyperpolarized by 6 mv, and the resistance fell 42 percent. The giant cell in B was depolarized by 9 mv, and the resistance fell 46 percent. Chloride conductance was doubled when pH fell from 8.0 to 5.0 (C), and potassium conductance was decreased by 25 percent (D).

cells tested, decreased external *p*H produced hyperpolarization, as would be predicted from the increased chloride conductance. In seven other cells, a^i_{Cl} was 40.7 ± 1.5 m*M*, with a range of 37 to 47 m*M*. Calculated E_{Cl} values were −53.3 ± 0.9 mv and ranged from −56 to −49 mv. For this group E_m was − 58 ± 0.8 mv, and, in two cells, decreased external *p*H evoked depolarization. This was also predictable from the increased chloride conductance and the relative values of E_{Cl} and E_m.

A somewhat parallel situation exists in the D (depolarizing) or H (hyperpolarizing) responses to acetylcholine shown by neurons of *Aplysia depilans* or *Helix pomatia*. In both species Cl conductance is increased, and the directional change of E_m is determined by E_{Cl} (*16*). In D cells of *Helix aspera*, $(Cl)_i$ was 27.5 m*M*, and in H cells it was 8.7 m*M* (*17*).

The reason for the two different levels of a^i_{Cl} is unknown. The giant cells showed hyperpolarizing responses (low a^i_{Cl}) throughout the year, whereas depolarizing responses (high a^i_{Cl}) were registered mainly in February and September. Strumwasser *et al.* (*18*) have reported a similar seasonal rhythm in the neural extract induction of behavioral egg-laying in *Aplysia*.

These experiments demonstrate that the effect of CO_2 depends upon the attendant fall in extracellular *p*H and not upon molecular CO_2 per se, decreased intracellular *p*H, or changes in bicarbonate ion concentration. Thus the addition of CO_2 at constant extracellular *p*H to the perfusate, a procedure which adds molecular CO_2 and bicarbonate ion and reduces intracellular *p*H, was without effect, whereas the addition of hydrogen ion in the form of a nonvolatile acid evoked the same effect as CO_2 and an equivalent fall in *p*H.

The increased chloride conductance elicited by a fall in *p*H has been reported (*19*). Such results are consistent with the classical idea of an amphoteric membrane whose selective permeability is due to the presence of fixed charges which allow passage of counter-ions and exclude co-ions. The dissociation of weakly ionizable groups will be altered by changes in external hydrogen ion, and this should result in a change of ionic permeabilities. At *p*H less than 7.5, the dissociation constants of the fixed charge groups are such that positive groups predominate and anion conductance increases. At higher *p*H, negative groups supervene and the passage of cations is facilitated.

The giant cells fell into two groups with respect to intracellular chloride activity. Those with low activities had $E_{Cl} > E_m$ and were hyperpolarized by CO_2; those with high activities had $E_{Cl} < E_m$ and were depolarized by CO_2. We propose, therefore, that the hyperpolarizing effect of CO_2 on cortical neurons and on phrenic and lumbar motoneurons, and the depolarizing effect on arterial chemoreceptors and respiratory center neurons, as well as the differences between several types of *Aplysia* neurons, can be explained by the same mechanism that produced hyperpolarization in some giant cells and depolarization in others. Thus, CO_2 causes a fall in extracellular *p*H which increases chloride conductance. If E_{Cl} for a given cell is greater than E_m, hyperpolarization ensues; if E_{Cl} is less than E_m, the cell is depolarized and an increased rate of discharge may follow.

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3 November 1969; revised December 1969

Line and Grade in the Extinct Medius Species Group of Sigmodon

Abstract. *Remains of* Sigmodon minor *and* Sigmodon medius, *extinct cotton rats comprising the* medius *species group, have been recovered from both Kansas and Arizona.* Sigmodon minor *evolved from* Sigmodon medius, *and the available evidence suggests that Kansas populations of* Sigmodon minor *were derived from Kansas populations of* Sigmodon medius, *while Arizona populations of* Sigmodon minor *were derived from Arizona populations of* Sigmodon medius. *This multiple origin of a single mammalian species is similar to the origin proposed by Carleton Coon for the races (subspecies) of* Homo sapiens.

Some concern has developed (*1*) over the somewhat unorthodox theory of the origin of human races presented by Coon in his book *The Origin of Races* (*2*). This theory was explained by Coon and crystallized further by Van Valen (*3*). Coon suggested a separate origin for each of five basic racial (subspecific) stocks of *Homo sapiens* (*sapiens* grade) from the same number of stocks of *Homo erectus* (*erectus* grade) in five separate world areas. He further suggested that evolution from the *erectus* grade to the *sapiens* grade need not have occurred simultaneously within all the evolving stocks.

Van Valen considered the above mechanism ". . . eminently plausible genetically" (*3*), but Montagu (*1*) considered it genetically implausible. Neither presented any evidence for their respective positions, which occasioned Montagu's pertinent query (*1*), "Where in the whole of animated nature is there