required to inhibit even highly dilute solutions of LDH-1 (*11*). Concentrations of NADH, rather than of pyruvate, are rate-limiting for the LDH reaction because under basal and particularly under anaerobic conditions NADH concentrations are appreciably lower than pyruvate concentrations. (iii) Inhibition of LDH-1 by pyruvate, though marked at 6° and 25°C, is greatly diminished at the physiological temperatures of many mammals (*3*, *11*, *17*).

If separate metabolic roles for LDH isozymes do exist, they are apparently based upon factors other than differential inhibition of LDH-1 and LDH-5 by pyruvate. Other metabolic roles recently suggested for LDH isozymes include (i) a regulatory function for LDH-5 which Fritz reported to be an allosteric protein (*18*); (ii) an association of isozymes in different proportions with various subcellular particles, in particular LDH-5 within the nucleus (*19*); (iii) predominance of LDH-5 in rapidly dividing cells or in tissues capable of rapid cell proliferation (*20*); and (iv) a conservative metabolic role in which one isozyme would be required to maintain critical enzymatic function in a tissue where another isozyme was rapidly degraded (*15*).

Our calculation of the LDH concentration in rat kidney reveals that in vivo LDH exists in concentrations several orders of magnitude above those customarily employed for the spectrophotometric assays in which pyruvate inhibition occurs. Those who have assayed LDH activity in mammalian tissue homogenates are acquainted with the requirement of a several hundredfold dilution before spectrophotometric measurement of the rate of NADH oxidation. We have previously suggested that an abortive ternary complex composed of LDH, pyruvate, and NAD may be responsible for the pyruvate inhibition of dilute concentrations of LDH (*6*). At low enzyme concentrations the abortive ternary complex forms rapidly, but it forms very slowly at physiologic LDH concentrations (*6*). This may explain why we did not detect substrate inhibition with high LDH concentrations and why substrate inhibition became pronounced at lower enzyme concentrations.

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2 JANUARY 1970

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10. The initial velocities measured were calculated to involve more than one catalytic turnover even at the highest LDH concentrations examined. In the 0.2-ml reaction mixture $7.0 \times 10^{-9}M$ LDH is equivalent to 1.4×10^{-6} mmole of LDH, which combine with 5.6×10^{-6} mmole of NADH, since each LDH molecule contains four binding sites for the coenzyme. At this concentration of LDH-5, the linear change in relative fluorescence intensity was 0.4 during the initial 50 msec of the reaction. This change corresponds to 4.5×10^{-5} mmole of NADH oxidized and indicates that eight catalytic turnovers have occurred within the 50-msec. interval.
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21. T.W. is the recipient of Public Health service fellowship 1 F2 HD-32, 763-01 from the National Institute of Child Health and Human Development.
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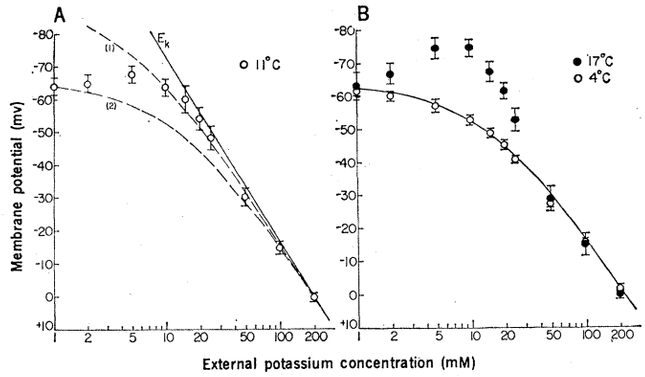
Membrane Potential as the Sum of Ionic and Metabolic Components

Abstract. *The resting potential of a molluscan neuron can be separated experimentally into two components: one which depends on ionic gradients and permeabilities in accordance with the Goldman equation, and a second which depends on the electrogenicity of active sodium transport.*

According to the ionic hypothesis (*1*), the neuronal resting potential results from ionic concentration gradients across a selectively permeable cell membrane. For some excitable cells, the potassium concentration gradient is primarily responsible for this potential, and the deviations of the membrane from the behavior of a simple potassium electrode have been accounted for by permeability of the membrane to other ions, such as sodium and chloride (*2*, *3*). However, to describe the resting potential of many excitable cells, different mechanisms of electrogenesis must be invoked or the ionic hypothesis modified (*4*, *5*). It has been hypothesized that the resting potential is generated by multiple mechanisms in a heterogeneous membrane (*6*). The questions may be asked whether a resting potential that is not predicted by the ionic hypothesis can be separated experimentally into components and whether a component exists that can be predicted by the ionic hypothesis. We report that the resting potential of a neuronal membrane is separable into at least two components: one which shows a classical dependence on ionic gradients and permeabilities, and a second which depends on temperature and metabolic activity.

Experiments were performed on the giant cell of the gastroesophageal ganglion of the marine mollusk *Anisodoris nobilis* (MacFarland) (*7*). This animal is generally found at water temperatures of 10° to 18°C, but may also live outside this range (*8*). The isolated ganglion was mounted in a linear chamber filled with flowing artificial seawater (478 m*M* NaCl, 10 m*M* KCl, 10.5 m*M* $CaCl_2$, 26 m*M* $MgCl_2$, 29 m*M* $MgSO_4$, and 2.5 m*M* $NaHCO_3$) which approximates the blood of the animal (*9*). Solutions were changed in less than 10 seconds. Test solutions were prepared by exchanging equivalent amounts of sodium and potassium, or by replacing sodium or chlo-



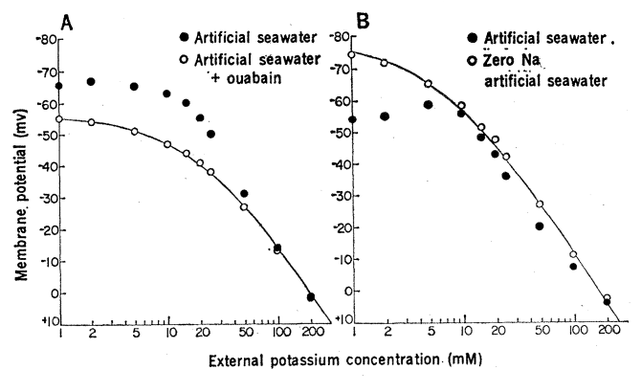


Fig. 1 (left). Dependence of the membrane potential on external potassium and temperature. Each point is an average, with S.E.M. shown, from five experiments. In (A) the temperature was 10.6° ± .4°C. Line E_K has the slope predicted by the Nernst equation for a K-electrode, $V = 56 \log (K_o/K_i)$; lines *1* and *2* were derived from Eq. 1 as indicated in the text. In (B), the temperatures were 17.4 ± .5°C (●) and 4.2 ± .4°C (○). The curved line was derived from Eq. 1 using the parameters $K_i = 235$, and $P_{Na}/P_K = .033$. Fig. 2 (right). Dependence of the membrane potential on external potassium before and after blockade of the sodium pump. The temperature was 11°C for both (A) and (B), and the curved lines were derived from Eq. 1. In (A), measurements were made before (●) and after (○) the addition of $10^{-4}M$ ouabain to the bath. In (B) measurements were before (●) and 30 to 60 minutes after (○) complete replacement of external Na with tris.

ride with an isosmotic equivalent of an impermeant ion such as tris (hydroxymethyl)aminomethane (tris) or SO_4. The temperature of the bathing solution was regulated with a thermo-electric device. Membrane potentials were recorded differentially between an intracellular and an extracellular microelectrode. The microelectrodes were filled with 3*M* KCl and were selected for resistance less than 10 megohms and tip potential less than 5 mv.

A semipermeable membrane whose potential is generated in accordance with the ionic hypothesis may be described empirically by the Goldman equation (*2*, *10*). Under conditions where chloride does not contribute significantly to the potential, and where $P_{Na}Na_i << P_KK_i$, this equation can be simplified to (*3*, *11*)

$$V = \frac{RT}{F} \ln \frac{K_o + (P_{Na}/P_K)Na_o}{K_i} \quad (1)$$

where V is the membrane potential; K_o, K_i, Na_o and Na_i are the external and internal concentrations of potassium and sodium, respectively; P_{Na} and P_K are the membrane permeabilities to sodium and potassium; R is the gas constant; T is the absolute temperature; and F is the Faraday constant.

Figure 1A shows the response of the membrane potential to different values of K_o at 11°C. Changes in potential were rapid, stable, and fully reversible at all values of K_o up to 300 m*M*, suggesting that K_i was not altered significantly during these brief tests (less than 10 minutes). Removal of external chloride caused little or no shift of the resting potential (less than 5 mv) and did not affect the magnitude or time course of the response to K, ruling out chloride as a major determinant of membrane potential. At high K_o, the resting potential depended largely on K, as shown by the proximity of the experimental points in Fig. 1A to the line labeled E_K, which has the slope predicted by the Nernst equation for a K-electrode. A somewhat better fit over the entire range of K_o was obtained by choosing values for P_{Na}/P_K and K_i in Eq. 1 so that the theoretical curve would intersect as many experimental points as possible (Fig. 1A). It is apparent, and can be shown mathematically, that no curve derived from Eq. 1 will encompass all the experimental points. In particular, the folding back or depolarization of the potential in low K_o cannot be explained by the Goldman equation unless either P_{Na} or P_K varies with the external ionic concentrations or some other process sensitive to K is superimposed on the ionic response of the membrane.

Agreement between the theoretical and experimental curves was altered by temperature (Fig. 1B). Cooling the giant cell to 4°C produced excellent agreement between the experimental points and a theoretical line derived from Eq. 1. Warming the cell to 17°C caused a greater hyperpolarization at $K_O = 10$ m*M* and a greater depolarization at low K_o than occurred on warming to 11°C. This hyperpolarization in normal K_o is approximately ten times that which would be predicted by the temperature dependence of Eq. 1, provided that K_i, P_{Na}, and P_K remain constant. The K_i estimated from the point of zero potential did not change significantly at different temperatures (Fig. 1).

The presence of a hyperpolarization on warming which was sensitive to K_o suggested that an electrogenic component of a Na pump might contribute directly to the potential of the cell (*12*). Experiments were run under conditions which should specifically block active Na efflux (*13*). In the presence of ouabain, or after depletion of internal Na by prolonged exposure to zero Na_o (tris) seawater, the membrane potential was accurately described by Eq. 1 at warm temperatures (Fig. 2). Thus, as suggested by Carpenter and Alving (*5*), an electrogenic component of the Na pump appears to be a primary factor in causing the temperature-sensitive deviation of the membrane potential from the Goldman equation. The presence of an important additional factor was indicated by the observation that when the Na pump was blocked with ouabain or zero K_o, warming caused a depolarization of the membrane rather than the small hyperpolarization predicted by Eq. 1. Removal of external Na eliminated this effect, suggesting that an increase of P_{Na}/P_K with increasing temperature might be responsible (*14*). The reduction of Na_o to zero did not eliminate the need for a "Na leakage" term in Eq. 1. Traces of Na may have been trapped in the extracellular space, the membrane may be slightly permeable to tris or other ions, or there may be other factors which prevent a cell from attaining unlimited potential.

The resting potential of the *Anisodoris* giant neuron can be separated into at least two components: one which can be predicted by the ionic hypothesis and another which depends upon the electrogenicity of active Na transport. This separation has special interest in light of reports that the Na pump not only makes a general contribution to potential, but may have more specific functions such as the mediation of synaptic effects (*15*). The ratio P_{Na}/P_K appears to vary with temperature and provides another mechanism by which the potential is regulated. A similar division of the resting potential into components may apply to excitable cells other than the *Anisodoris* giant neuron. Our data support the hypothesis (*6*) that the special characteristics of the resting potential in different nerve and muscle cells result from mechanisms, such as the Na pump, that modify a predictable ionic potential common to all excitable cells.

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Photooxidation of DDT and DDE

Abstract. *The pesticide DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane] and its metabolite DDE [1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene] can be photooxidized in methanol. Photolytic generation of free radicals that may abstract hydrogen from solvent, react with oxygen, or abstract hydrogen from unreacted substrate occurs. Further decomposition of short-lived intermediates yields many compounds. Oxidation products include benzoic acids, aromatic ketones, and chlorinated phenols. The DDE also undergoes photocyclization to give dichlorofluorene derivatives.*

The persistent insecticide DDT may be slowly degraded by the action of light and air. Photodecomposition of DDT has been discussed widely (*1*). We now report investigations of the photoxidation of DDT [1,1,1-trichloro-2-2-bis(*p*-chlorophenyl)ethane] and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene].

If nitrogen is bubbled through a methanol solution of DDT during irradiation, the radicals generated by photolysis abstract hydrogen from the solvent, and chlorine is lost. The rate of loss of chlorine from the trichloromethyl group of DDT at 2600 Å is shown in Table 1. Both DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] and 1-chloro-2,2-bis(*p*-chlorophenyl), ethane are obtained (Eqs. 1–4). The wavelength of the irradiation determines the nature of the products; at shorter wavelengths chlorine is displaced from the aromatic ring. Products of photodecomposition were identified by combined gas chromatography and mass spectrometry, as well as by other techniques (Table 2). The structures of the products support the sequence of reactions of free radicals postulated by Mosier *et al.* (*1*).

The products formed in the presence of oxygen are complex and result primarily from the reaction of oxygen with radical intermediates. A reaction sequence (Eqs. 5–8) is postulated for the formation of methyl-2,2-bis(*p*-chlorophenyl) acetate, a photooxidation product of DDT in methanol with oxygen (2800 Å).

The reaction sequence (Eqs. 5–7) follows that suggested for pentachloroethane photooxidation (*2*). A similar sequence could give rise to *p,p'*-dichlorobenzophenone (Eqs. 9–12). Solvent molecules may be involved in the reactions and add to the reactive inter-

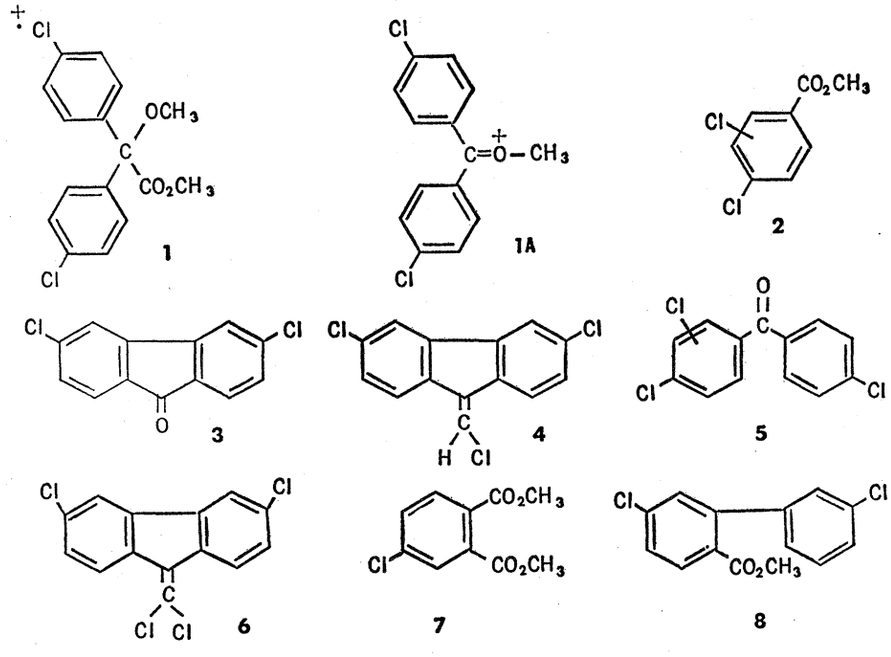

Fig. 1. Formulas of products of photooxidation.