perature (*9*). In each case the sharp transition to the superconducting phase in the annealed samples is almost certainly representative of the equilibrium properties of metallic gallium antimonide; it seems reasonable to conclude that the transition temperature is 4.24° ± 0.10°K.

The first change of the detector coil from the normal state on cooling at different magnetic fields can be taken as the definition of H_{c2}. So defined H_{c2} is 2640 gauss at 3.5°K for the annealed samples, and it decreases almost linearly with temperature to the transition.

The transition temperature for GaSb (4.24° ± 0.10°K) is higher than that for InSb [2.1°K (*5*)] or Sn (3.7°K) which have similar crystal structures. This trend is similar to that in the superconducting phases of Group III B elements—Tl, 2.36°K; In, 3.396°K; Ga(II), 7.5°K (*10*)—but opposite to that of Group V B elements—Bi(III), 7.25°K at 28 kb (*11*, *12*); Bi(II), 3.92°K at 25 kb (*12*); Sb, 2.6°–2.7°K (*13*).

D. B. McWhan
G. W. Hull, Jr.
Bell Telephone Laboratories, Inc.
Murray Hill, New Jersey
T. R. R. McDonald
E. Gregory
Central Research Laboratories,
Air Reduction Company, Inc.,
Murray Hill

References and Notes

1. S. Minomura and H. G. Drickamer, *J. Phys. Chem. Solids* **23**, 451 (1962); A. Jayaraman, W. Klement, Jr., G. C. Kennedy, *Phys. Rev.* **130**, 540 (1963).
2. J. C. Jamieson, *Science* **139**, 845 (1963).
3. T. R. R. McDonald, R. Sard, E. Gregory, *J. Appl. Phys.*, in press.
4. T. R. R. McDonald, G. S. Barberich, E. Gregory, paper 64-WA/PT-4, meeting of Amer. Soc. Mech. Engineers, New York, December 1964.
5. H. E. Bommel, A. J. Darnell, W. F. Libby, B. R. Tittmann, *Science* **139**, 1301 (1963); S. Geller, D. B. McWhan, G. W. Hull, Jr., *ibid.* **140**, 62 (1963).
6. We thank C. D. Thurmond for discussions on the properties of GaSb and for supplying the tellurium-doped gallium antimonide crystals.
7. A. L. Schawlow and G. E. Devlin, *Phys. Rev.* **113**, 120 (1959).
8. J. K. Hulm and R. D. Blaugher, *ibid.* **123**, 1569 (1961).
9. B. R. Tittmann, A. J. Darnell, H. E. Bommel, W. F. Libby, *ibid.* **135**, 1460A (1964).
10. W. Buckel and W. Gey, *Z. Physik* **176**, 336 (1963).
11. P. F. Chester and G. O. Jones, *Phil. Mag.* **44**, 1281 (1953).
12. N. B. Brandt and N. I. Ginzburg, *Zh. Eksperim. i Teor. Fiz.* **39**, 1554 (1960); English transl., *Sov. Phys. JEPT* **12**, 1082 (1961).
13. T. R. R. McDonald, E. Gregory, G. S. Barberich, D. B. McWhan, T. H. Geballe, G. W. Hull, Jr., *Phys. Letters* **14**, 16 (1965).
14. We thank T. H. Geballe for his interest and for helpful discussions, and G. S. Barberich and V. T. Cunicella for the preparative and x-ray work.

29 December 1964

Sodium Pump: Its Electrical Effects in Skeletal Muscle

Abstract. *The variations in the membrane potentials of skeletal muscle fibers which follow high rates of sodium extrusion are not due to changes in the ionic concentrations of the fiber; experiments suggest that sodium is extruded by an electrogenic mechanism.*

One of the problems in studying the mechanism by which ionic concentrations in nerve and muscle are maintained in a steady state at rest, in spite of the electrochemical gradient, is that of determining whether the sodium pump is electrically neutral or not.

The possibility of an electroneutral pump moving a potassium ion inward for each sodium ion that passes out was first suggested for red cells by Shaw (*1*) and by Hodgkin and Keynes (*2*) for nonmyelinated nerve fibers; a linkage between the fluxes of both ions has also been suggested for skeletal muscle (*3*). According to the results obtained by Kernan (*4*), Keynes and Rybova (*5*), and Mullins and Awad (*6*), however, the sodium pump is not electroneutral in skeletal muscle; Mullins and Noda (*7*) hold that if there is a linkage between the sodium and potassium pumps, the coupling ratio cannot be higher than one potassium ion pumped in for each three sodium ions pumped out; this makes the mechanism nonelectroneutral. In this report new evidence is presented in favor of the non-neutrality of the sodium pump in the skeletal muscle of the South American frog.

If there exists in the muscle membrane a potassium pump linked in some way to the sodium extrusion mechanism, the equivalent electric circuit of the membrane (*8*) may be expanded by adding two direct-current (constant or not) generators, representing the pumps, as shown in Fig. 1.

In such a circuit, the membrane potential difference $V_o - V_i$ may be altered by two mechanisms; (i) by changing the currents $i_{Na,p}$ and $i_{K,p}$ generated by the pumps; (ii) by causing a change in the electromotive forces E_K, E_{Na} and E_{Cl}, which represent, respectively, the equilibrium potential for K^+, Na^+, and Cl^-. If the two pumped currents compensate each other (the pump being neutral), the first mechanism by itself will not produce any change in the potential, but a change in the membrane potential will appear as soon as the rate of pumping is changed if the pump is

Table 1. Changes in membrane potential and extrusion of sodium.

Expt.	Extrusion of Na (mmole liter^{-1} min^{-1})	Membrane potential		Corrected potential (mv)
		At 3°C (mv)	At 25°C (mv)	
A	0.18	79	96	10
B	.50	46	92	42
C	.36	46	76	26
D	.29	63	86	17
E	.23	61	76	9
F	.24	63	77	8
G	.15	61	73	6
H	.11	63	77	8

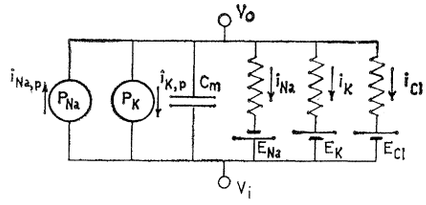

Fig. 1. Equivalent electric circuit of the membrane. E_{Na}, E_K, and E_{Cl} are the equilibrium potentials of Na^+, K^+, and Cl^-, respectively; C_m is membrane capacity; i_{Na}, i_K, and i_{Cl} are the passive currents of Na^+, K^+, and Cl^-. P_{Na} and P_K are ion permeabilities; $i_{Na,p}$ and $i_{K,p}$ are the currents generated by the Na and K pumps; V_o and V_i are potentials outside and inside the membrane.

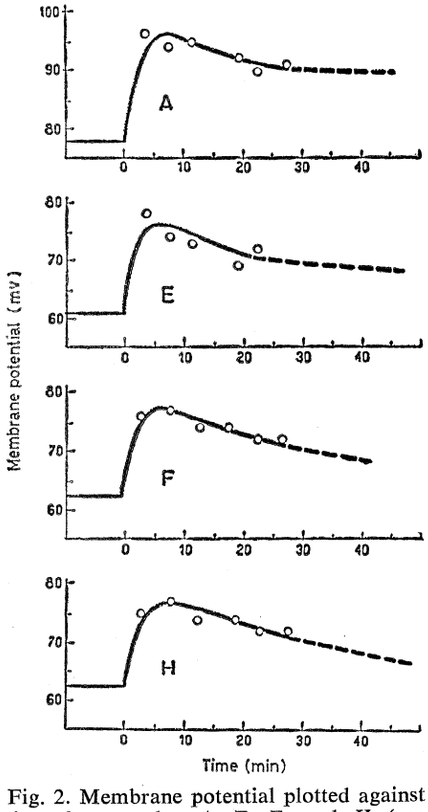

Fig. 2. Membrane potential plotted against time for muscles *A*, *E*, *F*, and *H* (see Table 1). Each point is a mean of 10 to 12 punctures.

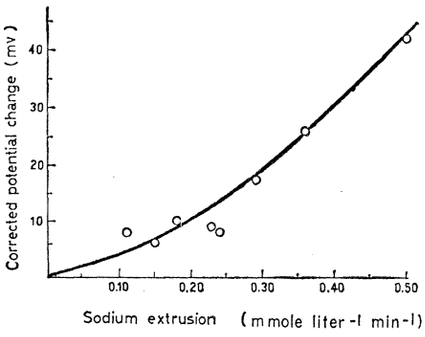

Fig. 3. Changes (corrected) in potential plotted against sodium extrusion. Circles represent values taken from Table 1.

electrogenic. The second mechanism will always lead to a change of the membrane potential (regardless of pump neutrality), but only because of changes in ionic concentrations produced by the pump. Such an alteration requires time for significant changes in ion concentrations to occur. According to this line of reasoning, the mechanism by which membrane potentials are generated may be deduced by recording the change in membrane potential with time in response to changes in the rate of ion pumping. Change in the rate of pumping can be initiated by change in temperature (*9*).

Sartorius muscles of the South American frog, *Leptodactylus ocelatus*, were dissected and kept overnight in the cold (3°C) in potassium-free Ringer's solution (per liter: Na^+, 120 mmole; Ca^{++}, 1.8 mmole; Cl^-, 123.6 mmole) to increase the internal sodium concentration of the fibers. Next morning, both muscles of the same pair were transferred to normal Ringer solution (per liter: Na^+, 120 mmole; K^+, 2.5 mmole; Ca^{++}, 1.8 mmole; Cl^-, 126.1 mmole) at 3°C, and the membrane potential was recorded by the microelectrode technique (*10*). One of the muscles (*A*) was separated in a crucible for analysis, while the other (*B*) was kept in the bathing solution. The temperature of the bath was then raised as rapidly as possible to 25°C, and the potential of the membrane was recorded for 1 hour by repeated puncturing of different muscle fibers. The muscle was then taken from the bath and both muscles were analyzed for sodium by flame photometry. From the initial concentration (of muscle *A*) and the final one (of *B*) and the time elapsed, an estimate was made of the amount of sodium extruded by the second muscle per unit time during the period at 25°C. Some examples of the curve obtained when potential is plotted against time are shown in Fig. 2, where the temperature was changed at time zero. As the temperature changed, the potential of the membrane increased. The rise was rather steep at the beginning, but after a few minutes it reached a maximum and then declined slowly. A maximum potential appeared in all the experiments, and the change in the potential ΔV was always higher than the change in potential that would be obtained by changing temperature T in the equation for the potential of a membrane (*11*)

$$V = \frac{RT}{F} \log_e \frac{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}$$

In this equation R is the gas constant, F the Faraday constant, P_K, P_{Na}, and P_{Cl} are, respectively, the permeabilities for K^+, Na^+, and Cl^- and the symbols within brackets represent concentration; subscripts o and i indicate, respectively, outside and inside. After 20 to 30 minutes the result showed considerable variation, although in all the experiments there was a tendency for the potential to decline, as shown by the dashed lines in Fig. 2. Table 1 shows the changes in sodium concentration per unit time, as well as the potentials obtained from several experiments. The last column gives the maximum corrected potential with the effect of temperature taken into account; that is, the maximum potential change observed minus the change obtained by introducing the same temperature change in the above equation. Figure 3 shows the corrected changes in potential plotted against sodium extrusion per unit time. It is possible to draw a line passing through the origin, which means that if there is no sodium extrusion, the potential does not change. Evidently, there is a direct relation between sodium extrusion and potential change, although it is not yet possible to establish a function that relates both variables.

The curves in Fig. 2 show that the maximum change in potential occurs in a time so short that internal ion concentrations have not changed. For example, in experiments E and F (see Table 1) the change in the potassium concentration in 6 minutes might be 1.5 mmole/liter at most, which is not enough to explain a potential change of about 8 mv observed within that period. With respect to chloride, if it is assumed that it is passively distributed, its concentration is a consequence rather than a cause of the changes in potential. If, on the contrary, the pump were not neutral and generated an outward current, the difference in potential across the membrane would increase until the passive currents of chloride and potassium would compensate its effects. This would occur within the first 10 minutes at the maximum height of the curves. The final decline of the curves might be explained by a decrease in the rate of the pump as the concentration of sodium decreases.

ANTONIO S. FRUMENTO
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

References and Notes

1. I. M. Glynn, *Progr. Biophys. Biophys. Chem.* **8**, 241 (1957).
2. A. L. Hodgkin and R. D. Keynes, *J. Physiol.* **128**, 28 (1955).
3. R. D. Keynes, *Proc. Roy. Soc. London Ser. B* **142**, 359 (1954); ——— and R. C. Swan, *J. Physiol.* **147**, 591 (1959).
4. R. P. Kernan, *Nature* **193**, 986 (1963).
5. R. D. Keynes and R. Rybova, *J. Physiol.* **168**, 58P (1963).
6. L. J. Mullins and M. Z. Awad, *J. Gen. Physiol.*, in press.
7. L. J. Mullins and K. Noda, *J. Gen. Physiol.* **47**, 117 (1963).
8. A. L. Hodgkin and A. F. Huxley, *J. Physiol.* **117**, 500 (1952).
9. R. D. Keynes, *Proc. Roy. Soc. London Ser. B* **142**, 359 (1954).
10. J. Graham and R. W. Gerard, *J. Cellular Comp. Physiol.* **29**, 99 (1946).
11. A. L. Hodgkin and B. Katz, *J. Physiol.* **108**, 37 (1949).

12 November 1964

Uracil Mustard: A Potent Inducer of Lung Tumors in Mice

Abstract. *Uracil mustard, injected intraperitoneally, increased the incidence and average number of pulmonary tumors in A/J mice. In comparison to a typical alkylating agent, nitrogen mustard, or to urethan, uracil mustard (on a molar basis) was considerably more carcinogenic.*

The carcinogenic polycyclic aromatic hydrocarbons (*1*), urethan (*2*, *3*), and both sulfur mustard and nitrogen mustard (*4*) increase the incidence and average number of pulmonary tumors in inbred strains of mice. This fact is the basis of an assay of carcinogens that has been used in numerous investigations to study the effects of strain, age, diet, sex, and other factors (*5*). The system is useful because of the relatively short latent period before tumors arise. Also the assay may be quantitatively evaluated in both the percentage of the tumor-bearing mice and in the number of individual tumor nodules in the lungs. These two criteria permit accurate correlation of dosage to response and of chemical structure to activity.