Although it is certainly possible that a more severe vitamin B_6 deficiency would have resulted in impaired L-alanine transport it is doubtful that significantly greater depletion of pyridoxine can be produced in the rabbit by dietary restriction. We are forced to conclude that the ability of *in vitro* segments of rabbit ileum to transport L-alanine is not affected by chronic, severe pyridoxine deficiency.

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Department of Obstetrics and Gynecology, R. A. CHEZ
University of Pittsburgh School of Medicine, E. O. HORGER, III
Pittsburgh, Pa. (U.S.A.)
Department of Physiology, STANLEY G. SCHULTZ
University of Pittsburgh School of Medicine,
Pittsburgh, Pa. (U.S.A.)
Department of Physiology, P. F. CURRAN
Yale University School of Medicine,
New Haven, Conn. (U.S.A.)

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An estimation of the sodium and potassium equilibrium potentials in the muscle membrane of the earthworm, *Pheretima hawayana* R.

The South American earthworm, *Pheretima hawayana* R.[1], has been found to have a low resting potential, E_{m}, across its muscle membrane, maintained by the membrane conductances to more than one monovalent ion[2–4], spontaneous diphasic reversals of the E_{m} (refs. 2–4) and no electrical excitability[4]. In order to analyze these observations, the membrane behavior in response to the ionic environment, especially to that of Na^+ and K^+, must be studied. This report is limited to the estimation of the theoretical equilibrium potentials caused by the passive distribution of these two ions across the membrane. Other important features, such as their resting flux rates and conductance change during potential reversal, are not dealt with here.

The intra- and extracellular concentrations of Na$^+$ and K$^+$ were calculated by measuring the ionic contents of the whole tissue and the extracellular space. The extracellular concentrations were taken as those of the Ringer solution modified[2] from that used for the Northern Hemisphere species *Lumbricus terrestris* L. The muscle preparation was dissected out as previously described[2]. All the preparations were quickly rinsed in deionized water and blotted dry before being used in the experiments. The weight/volume ratio of the wet tissue was 0.939 ± 0.096 g/ml; this was taken as 1.0 g/ml for convenience. The total ionic contents of 20 preparations were determined by spectrophotometry after incineration. The weight of the ash was 0.01% of the wet tissue and was assumed to be negligible. The total K$^+$ content was 35.3 ± 3.9 mmoles/kg, and the total Na$^+$ content was 41.4 ± 4.0 mmoles/kg. The extracellular space was measured in 16 preparations by the sodium ferrocyanide method[5,6]. Fig. 1 shows a graph of the space occupied by sodium ferrocyanide per kg wet tissue plotted against the incubation time. A maximum volume of 0.2 l/kg was reached after incubation for 1 h, and this was maintained until the incubation time exceeded 3.5 h, when the space again began to increase, probably indicating tissue swelling. This was confirmed by examining a frozen section (10 μ thick) of the preparation after incubation for 1 h in sodium ferrocyanide; this compound did not penetrate into the intracellular space. The intracellular potassium concentration, $[K^+]_i$, was calculated as 43.1 ± 4.9 mM by assuming that the extracellular space, filled with 4 mM potassium[2], is 20% of the total, and that the remaining 80% of the space is intracellular. The intracellular sodium concentration, $[Na^+]_i$, was calculated as 17.2 ± 5.0 mM on the same basis (the value of $[Na^+]_0$ was taken[2] as 138 mM). The potassium equilibrium potential, E_K, across the resting membrane, was estimated to be —59.9 mV, from inside to outside, by substituting the intra- and extracellular potassium concentrations into the Nernst equation. The sodium equilibrium potential, E_{Na}, was estimated to be 52.4 mV by the same method (Fig. 2). The E_m and the spontaneous spikes were recorded by intracellular microelectrodes. The E_m had the same magnitude (37.3±0.4 mV in 20 cells) as in the previous report[2].

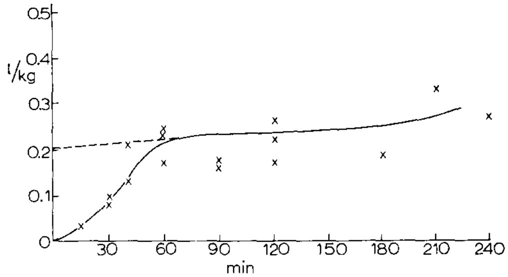

Fig. 1. Determination of intracellular space by the ferrocyanide method. Abscissa shows incubation time. Ordinate shows the space supposed to be occupied by sodium ferrocyanide. Dotted line shows the intersection on the ordinate of the volume of the space when stable conditions were attained.

It can be seen from Fig. 2 that the recorded E_m was only about two thirds of the calculated E_K. This is in agreement with the previous suggestion[2] that the E_m

is not maintained by potassium conductance (g_K) alone, as in the case of frog skeletal muscle and the squid axon. The so-called after-potential[2] of the spontaneous spike (Fig. 2) is probably comparable to the positive phase of a diphasic discharge[7–9] which is related to potassium activation. It has indeed been observed that the peak of the positive phase approaches E_K and frequently undershoots it when $[K^+]_0$ is at its normal value. The observation[2] of abolition of the spontaneous discharge at $[K^+]_0$ values above 24 mM supports this view, since the calculated E_K at this value of $[K^+]_0$ would be too low for activation. The use of the $[K^+]_0$ and $[Na^+]_0$ values taken from the Ringer solution used with *Lumbricus terrestris* L., for calculation in this work may be subject to question, since it has been found that membrane electrogenesis phenomena are profoundly different even in two closely related species[3,4]. However, indirect evidence from previous work indicates that the assumed values of $[Na^+]_0$ apply to this species as well, since the E_m of this species is sensitive to $[Na^+]_0$ variations (Fig. 3A of ref. 2). A large deviation in the value of $[Na^+]_0$, but not $[K^+]_0$, would easily be detected in this species by its influence on the E_m.

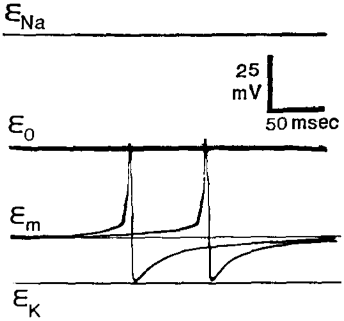

Fig. 2. Diagram showing the relation of calculated sodium equilibrium potential, E_{Na}, and the calculated potassium equilibrium potential, E_K, to the recorded E_0 (zero level potential), E_m (resting potential) and two spontaneous spikes.

Department of Physiology,
State University of Paraná,
Curitiba (Brazil)

Y. C. CHANG
L. RITZMANN

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