In the course of our work we observed that the enzyme is induced in intact rats under the conditions of anesthesia and surgery without the administration of the glucocorticoid, presumably under the influence of a stress-mediated release of corticosterone. A five- to eightfold induction can thus be effected. Figure 2A illustrates the action of cycloheximide in such animals. The stress-mediated induction is again inhibited by the antibiotic. Without inclusion of the right controls the small elevations in tyrosine transaminase levels noted in the cycloheximide-treated animals would resemble a very modest enzyme "induction." In fact, they represent residual activities of the stress-mediated induction which is largely inhibited by the antibiotic.

Figure 2B illustrates the effect of cycloheximide on the induction of tyrosine transaminase by hydrocortisone in intact anesthetized rats. In agreement with Rosen *et al.* (*11*), we found that the response of the enzyme to hydrocortisone in these animals is lower than that in adrenalectomized animals. The kinetics of the induction and its inhibition by cycloheximide are quite similar to those established in the adrenalectomized rats (Fig. 1).

Our experiments demonstrate clearly that cycloheximide is not an inducer of tyrosine transaminase in the anesthetized rat and, rather than enchancing (*3*) the action of hydrocortisone, cycloheximide inhibits the inductions caused by the glucocorticoid in both intact and adrenalectomized rats as well as the stress-mediated induction in the intact animals. The significance of the small, erratic elevation of tyrosine transaminase noted in some animals (Table 1) is unclear. Because of the erratic nature of the response in these animals, further speculation on the possibility of differential inhibition of repressor formation referred to in the beginning of our report is not justified at this stage. It must be noted that in the report by Fiala and Fiala (*3*) elevations of the enzyme in cycloheximide-treated adrenalectomized rats are tabulated for only three animals against one control. Inclusion of more animals in this type of experiment might have resulted in eliminating a large part of the discrepancy between their results and ours. We have observed that large variations in basal levels of tyrosine transaminase do occur in adrenalectomized rats.

In conclusion, the speculation (*3*) that the reported elevations of tyrosine transaminase may represent a "pseudohormonal" induction in which hydrocortisone is replaced by cycloheximide for the induction of the enzyme is not consistent with our experimental data gathered by the use of the serial biopsy technique.

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Calcium-Selective Electrode with Liquid Ion Exchanger

Abstract. *A simple calcium-selective electrode has been developed which is capable of measuring calcium ion activity in the presence of many common interfering ions. The electrode utilizes a liquid ion exchanger membrane containing the calcium salt of a disubstituted phosphoric acid.*

At the present time there are no ion-sensitive electrodes which have a sufficiently high selectivity for calcium ion in the presence of sodium and other monovalent cations to permit their use in the analysis of biological fluids for calcium ion activity. Typical biological samples have monovalent cation ion concentrations on the order of 100 times the concentration of uncomplexed calcium ion. This report describes a liquid ion exchange electrode capable of measuring free calcium ion activity in the presence of a 1000-fold excess of sodium or potassium ions.

The electrode was constructed from a glass tube, 1 cm in diameter, sealed at the lower end with cellulose dialysis tubing. The tube is filled to a depth of approximately 2 cm with a liquid ion exchange solution, 0.1*M* in the calcium salt of didecylphosphoric acid dissolved in di-*n*-octylphenyl phosphonate. Electrical contact is made to the organic phase via a narrow glass tube containing a 0.1*M* $CaCl_2$ aqueous 2 percent agar gel and a silver–silver chloride electrode. Potential measurements were made with a Corning model 12 *p*H meter.

The water immiscible organic phase in the tube forms a liquid ion exchange "membrane" whose ion exchange properties for cations are similar, in mechanism, to the liquid ion exchanger concentration cells reported by Sollner (*1*) and Bonner (*2*). To the extent that calcium is the only cation present in the sample which can participate in ion exchange with the organic calcium salt, then charge transport between the sample and the internal agar phase involves net movement of calcium ions only; that is, the transport number of calcium ions across the organic phase is unity. Under these conditions the equilibrium potential across the membrane is given by the Nernst equation:

$$E_{\text{memb.}} = \text{constant} + \frac{RT}{2F} \log A_{Ca^{++}} \quad (1)$$

where $A_{Ca^{++}}$ is the "ionic activity" of calcium ion in the aqueous sample phase. The value of the constant term depends on the particular choice of reference electrode and the activity of calcium ion in the internal agar phase.

It was first necessary to establish that the calcium electrode behaved in an ideal Nernst manner under conditions where no assumptions regarding ionic activity coefficients or liquid junction potentials were necessary. Table 1 shows data obtained with the cell

Ca electrode | $CaCl_2$ (*M*) | AgCl | Ag

Provided that the calcium electrode functions as a Nernst electrode for calcium ions the cell potential should be given by

$$E_{\text{cell}} = \text{constant} + \frac{3RT}{2F} \log M\gamma_{\pm} \quad (2)$$

where $\gamma_{\pm}$ is the mean ionic molal activity coefficient of calcium chloride and M is the molal concentration. The value of the constant depends on the activity of both calcium and chloride ions in the internal agar gel. Over a 4-decade concentration range from 10^{-4} to 1*M* an E_{cell} versus log $M\gamma_{\pm}$

plot can be fitted by a straight line with a slope of 87.5 mv, in good agreement with the slope of 87.9 mv predicted from Eq. 2. Below $10^{-4}M$ the potential deviates in a positive sense, becoming essentially independent of concentration below $10^{-6}M$ calcium chloride. This low concentration limit is set by the solubility of the organic calcium salt in the aqueous phase. Above $1M$ considerable scatter occurs in the data. The cause of this is unknown but it may be due to the difficulty of obtaining equilibrium at the Ag | AgCl electrode at very high chloride concentrations.

The practical utility of a calcium-selective electrode depends on its ability to respond to ion activity in the presence of other ions using a cell of the type

Ca electrode | sample solution | liquid junction | saturated KCl-calomel reference electrode

A rigorous evaluation of electrode selectivity in solutions of practical interest is difficult, owing to uncertainties in the reference electrode liquid junction potential and the questionable ability of the Debye-Huckel equation to predict ionic activity coefficients in mixed ion solutions at high ionic strength.

Typical behavior of the cell in mixed ion solutions is shown in Fig. 1 for the ion pair Ca^{++}–H^+ in chloride solution. The effect of foreign cations in electrode potential for all ions investigated can be fitted by the following empirical equation:

$$E = \text{constant} + \frac{RT}{2F} \log [A_{Ca^{++}} + \sum_i k_i A_i^{2/Z_i}] \quad (3)$$

where A_i and Z_i refer to the activity and charge of an interfering ion i. The selectivity constant k_i is a measure of the degree to which the ion interferes with the measurement of calcium ion activity. Values of k obtained from measurements in mixed ion solutions and Eq. 3 are: $k_{H^+} = 10^5$, $k_{Na^+} = k_{K^+} = k_{NH_4^+} = 10^{-4}$, $k_{Mg^{++}} = 0.014$, $k_{Ba^{++}} = 0.010$. The ionic activity coefficients used in calculations of k_i were obtained from an extended Debye-Huckel equation (*3*). Values of k_i depend to some extent on solution composition but are constant to ± 20 percent over variations in calcium concentration of from 10^{-4} to $10^{-1}M$ and 10^{-2} to $1M$ in the interfering ion.

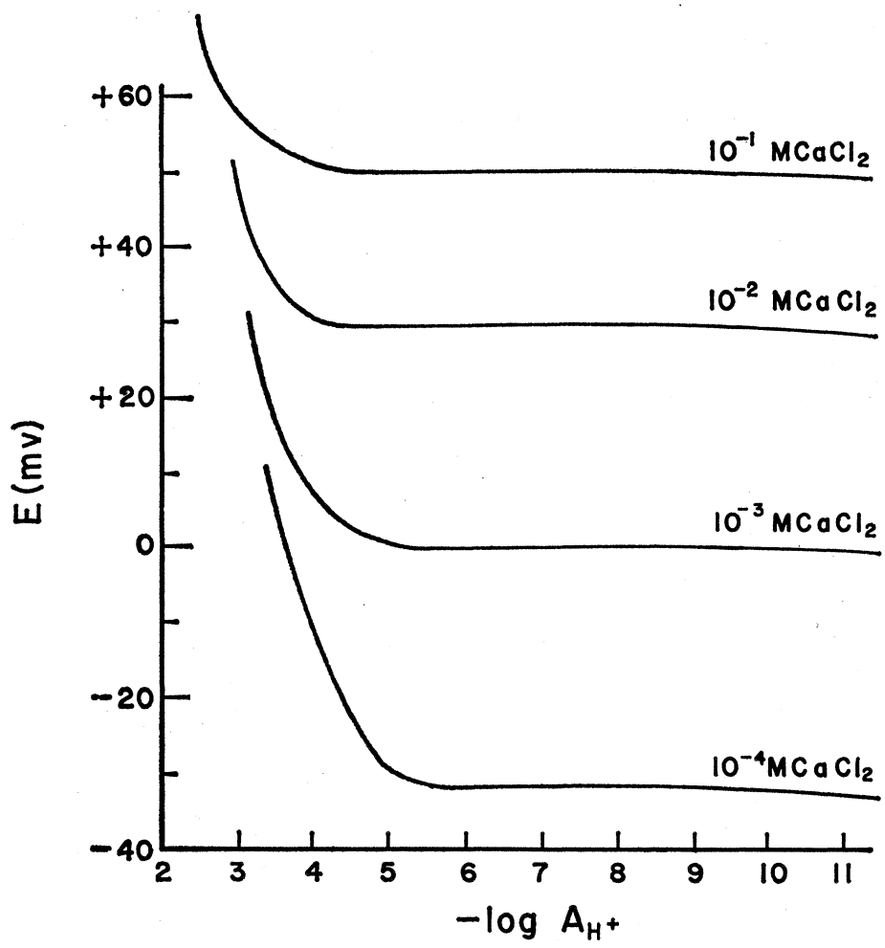

Fig. 1. The effect of hydrogen ion interference on calcium electrode potential at various calcium concentrations. The *p*H was varied with small increments of NaOH and HCl.

Table 1. The potential of the cell Ca electrode | $CaCl_{2(M)}$ | AgCl | Ag at 25°C. The mean ionic activity coefficients at higher concentrations have been taken from Frankenthal (*3*). At lower concentrations a Debye-Huckel calculation has been used.

M (moles per liter)	$\gamma_\pm$	E_{cell} (mv)
3.94	4.29	210.5
1.70	0.742	156.5
8.65×10^{-1}	.490	102.5
8.83×10^{-2}	.525	14.2
8.83×10^{-3}	.738	− 60.2
8.83×10^{-4}	.897	− 140.6
8.83×10^{-5}	.96	− 223.7
2.65×10^{-5}	.98	− 264.0
8.83×10^{-6}	.99	− 287.2

In general the nature of the anion present in the sample solution has no effect on electrode response other than lowering the calcium ion activity through complexation or ion pair formation. Exceptions may be expected for those anions which form calcium salts with high solubilities in low dielectric constant organic solvents. Of the common inorganic anions studied to date only perchlorate ion appears to interfere seriously in calcium activity measurements.

The time response of the electrode on changing sample solutions is rapid, usually less than 30 seconds provided the sample solutions do not contain large concentrations of interfering cations. Time response in solutions where the terms k_iA_i for interfering ions are of the same order of magnitude as the activity of the sample calcium may be as long as 10 minutes.

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