failure. Moreover, Feinstein has shown that cardiac function can be normal in spite of a considerable depression of high energy phosphate stores(14). Several other studies have shown that ischemic heart failure will ultimately result in a reduction of myocardial ATP stores(4-8). However, the results of the present study show that there is no detectable alteration in myocardial ATP stores at the onset of ischemic heart failure. Thus it would appear from the present investigation and an earlier study on acute hypoxic heart failure carried out in this laboratory(8) that reduction of O_2 delivery to the myocardium can impair cardiac function without lowering total myocardial ATP levels.

The results of the present study would be compatible with a defect in energy storage as the cause of ischemic heart failure only if a very small undetectable fraction of the total ATP store, such as that used for the maintenance of membrane function, were depleted (8,15,16). On the other hand ischemia might interfere directly with excitation-contraction coupling or with the myofilaments themselves and in this manner alter the mechanical performance of the myocardium.

Summary. The effects of acutely induced ischemic heart failure on myocardial high energy phosphate stores were studied in dogs following an abrupt reduction (avg. 40%) of left main coronary arterial flow. Left ventricular biopsies were obtained before and during the onset of heart failure. Myocardial ATP stores were unchanged (control, 6.65 μmoles/g; ischemia, 6.66 μmoles/g) at a time when left ventricular end-diastolic pressure had risen from 3.9 to 11.3 mm Hg and left ventricular stroke work had fallen from 7.9 to 4.9 g-m. The average myocardial creatine phosphate (CP) stores fell from 13.2 μmoles/g to 7.9 μmoles/g. These results indicate that acute ischemic heart failure is not initiated by a detectable depression in total myocardial ATP stores.

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Complexometric Titrations Using Calcium Specific Electrodes.* (31682)

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Ionized calcium is believed to be essential for the execution of a number of physiological functions which include muscle contraction, blood clotting, and nerve impulse transmis-

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sion. The chemistry of agents which bind calcium is therefore also of great importance to biology. The binding of ionized calcium by chelating agents is studied by carrying out complexometric titrations.

Complexometric titrations involving calcium have frequently been carried out in the past using physical properties that depend on the ligand properties such as optical density in the ultraviolet region(1), pH variation(2) or nonspecific physical properties of the calcium ion such as conductivity(3). With the advent of the calcium specific electrode recently, it has become possible to follow the ionized calcium concentration directly during titration with a ligand.

Methods. The calcium electrode used in these studies is commercially available from the Corning Glass Works, Medfield, Mass. The potential difference generated by putting this electrode in a calcium solution was determined against a standard calomel electrode using a Radiometer Model 4 potentiometer.

Discussion. Before complexometirc titrations could be carried out it was necessary to establish the relationship between the potential of this electrode relative to a standard calomel electrode and the calcium ion concentration, the sodium ion concentration and the hydrogen ion concentration, as well as the interaction of these 3 parameters. The initial problem was to establish the range of calcium ion concentration to which this electrode has a linear response. As is shown in Fig. 1, this electrode gives a linear relationship between potential in millivolts and the log of the calcium ion concentration for values of calcium ion concentration 10^{-1} M calcium ion to 10^{-5} M calcium ion. Below 10^{-5} M calcium ion this electrode no longer gives such a linear relationship. Potential values obtained from pure calcium chloride in distilled water do not differ from those of the same concentration of pure calcium chloride in 10^{-2} M sodium cacodylate buffer.

The independence of the potential of the electrode with respect to sodium ion is shown in Fig. 2. The sodium ion concentration was varied from 1 M to 10^{-3} M over 3 decades of concentration of calcium ion, and in no

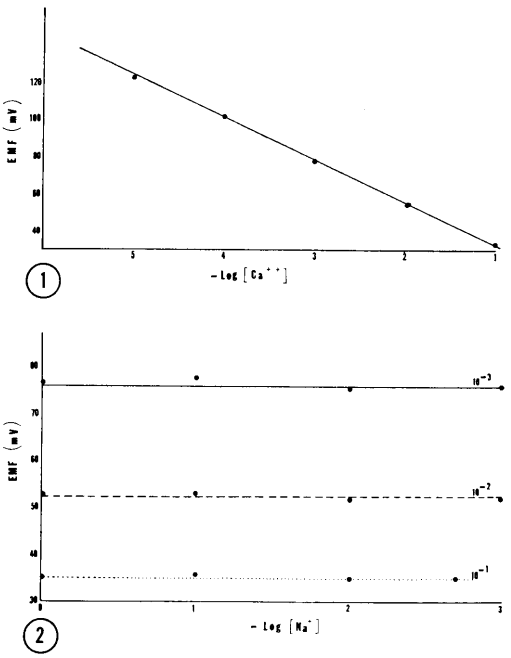

FIG. 1. Linear relationship between the potential and the log of calcium ion concentration over 5 decades of calcium ion concentration. Potential values are the same if the calcium is dissolved in distilled water or 10^{-2} M sodium cacodylate buffer.

FIG. 2. Independence of potential of the electrode with respect to sodium ion over 4 decades of sodium ion concentration and 3 decades of calcium ion concentration. Dotted line represents 10^{-1} M calcium ion, dashed line represents 10^{-2} M calcium ion and solid line represents 10^{-3} M calcium ion.

case was the potential of the electrode found to vary with the sodum ion concentration. It is therefore concluded that it would not be necessary to make corrections for the changes in sodium ion concentration that occur during the titration.

The marked dependence of the electrode with respect to the hydrogen ion concentration is shown in Fig. 3. From pH 7 to pH 5, the potential of the electrode was found not to vary with pH over 3 decades of calcium ion concentration. Within this pH range, the linear dependence of potential on the log of the calcium ion concentration was found to hold true.

Between pH 5 and pH 4 the potential values suddenly become more negative. Below pH 4 this trend reverses itself and by 10^{-1} M hydrogen ion, the calcium electrode is actually positive with respect to the saturated

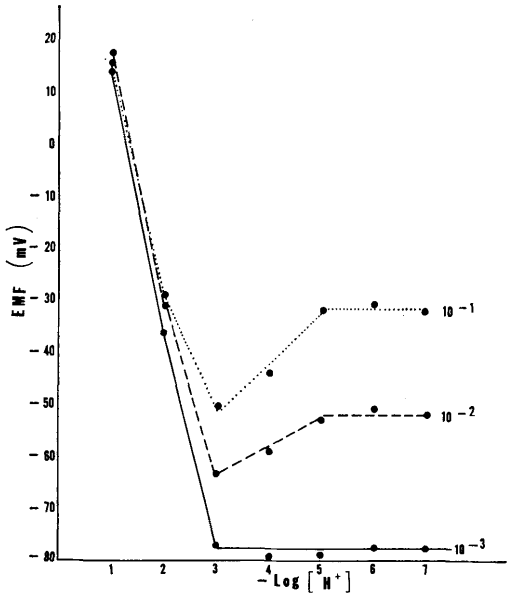

FIG. 3. Marked dependence of electrode potential on hydrogen ion concentration. Below pH 5, electrode does not respond linearly with the log of calcium ion concentration. Dotted line represents 10^{-1} M calcium ion, dashed line represents 10^{-2} M calcium ion, solid line represents 10^{-3} M calcium ion.

calomel electrode. Concomitant with this pH dependence below pH 5, the electrode no longer exhibits a linear dependence of the potential with respect to the log of the calcium ion concentration. Thus, this electrode could be used only with difficulty, if at all, below pH 5 to measure the calcium ion concentration.

Because of the high stability of the calcium-ethylenediamine tetraacetate $(CaEDTA)^{-2}$ complex it was decided to use as a model complexometric titration, the titration of a calcium chloride solution with a sodium ethylenediamine tetraacetate (Na_2EDTA) solution both buffered at pH 7.0 with sodium cacodylate buffer. The results of such a titration are shown in Fig. 4 where the negative logarithm of the calcium ion concentration is plotted against the ratio of total moles of EDTA to total moles of $CaCl_2$. The first and somewhat surprising result of this titration is that initially, for every mole of EDTA added, approximately 2 moles of calcium ion are complexed. However, it is also obvious that the end point of this titration occurs when there are an equal number of moles of EDTA and calcium ion. From these results it is apparent that there are two different complexes of EDTA with calcium; the first has the stoichiometry Ca_2EDTA *i.e.*, the 2:1 complex and the second has the stoichiometry CaEDTA *i.e.*, the 1:1 complex. Previous titrations(2,4) have all calculated the concentration of calcium ion assuming that the stoichiometry of the complex was CaEDTA, whereas the present direct measurement unequivocally shows that this assumption was not warranted. A more complete discussion of the stoichiometry, free energy of formation, enthalpy of formation, and entropy of formation together with the appropriate stability constants will be published.

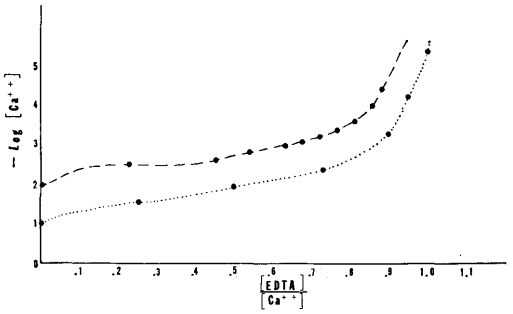

FIG. 4. Results of a complexometric titration of calcium ion with EDTA at pH 7. At all points there is evidence of formation of CaEDTA, and Ca_2EDTA is also formed. Dotted line represents titration of 10^{-1} M calcium ion with 10^{-1} M EDTA. Dashed line represents titration of 10^{-2} M calcium ion with 10^{-2} M EDTA.

Summary. This new technique is valuable in 3 different areas: the quantitative determination of calcium ion concentration, unequivocal determination of the stoichiometry of a complex, and determination of stability constants for complexes of various ligands with calcium ion. These cation specific electrodes are extremely useful in the study of chelation phenomena in biochemical reactions.

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