samples below −100°C in the microscope vacuum. An advantage of this procedure is that the water content of subcellular compartments can be measured directly.

Some investigators choose instead to vacuum freeze-dry their tissue. Although some ion movement may occur during drying, as ions migrate to find counterions, such movement seems to be fairly restricted. Freeze-dried material is easier to handle, and gives better image contrast and higher X-ray signal-to-noise ratios, but information about subcellular water content is lost.

A technique which combines the advantages of ultrarapid freezing with the high resolution and convenience of conventional embedding, is freeze-substitution in acetone and acrolein[4]. After the tissue is frozen, acetone is substituted for the ice over a period of days. Acrolein, added to the acetone, improves structural preservation. At present, this relatively new method appears to be suitable only for calcium, and although very little calcium is lost from the tissue, some question remains as to the extent of its intracellular redistribution.

Applications

The first applications of X-ray microanalysis were in metallurgy and mineralogy. But biologists soon grasped its potential as a probe of elemental composition in cells. Today, X-ray microanalysis is applied in physiology, cell biology, pathology, clinical medicine, and many other areas of biological and biomedical research. We cite the following two studies to illustrate the use of this technique in investigation of subcellular elemental changes.

Somlyo *et al.*[5] studied calcium release from the sarcoplasmic reticulum of tetanized skeletal muscle. By probing the terminal cisternae of resting and tetanized muscle, they were able to directly show that 59% of the calcium was released into the cytoplasm during a 1.2 second tetanus, and that this was accompanied by an increase in cisternal magnesium and potassium.

This application of X-ray microanalysis exploits the unique ability of the method to detect milimolar concentration changes in small subcellular compartments. These results complement previous studies with other methods which have concentrated on monitoring changes in cytoplasmic free calcium presumed to result from the release of calcium from the sarcoplasmic reticulum.

In our laboratory we have recently completed a study of changes in the elemental composition of sperm during and following induction of the acrosome reaction[6]. Previous studies using radioisotopes and ion-sensitive electrodes had demonstrated that ion fluxes occur[7]. However, the magnitude and intracellular location of the changes was not known and only Na, K and Ca had been studied. Using X-ray microanalysis, we quantified cytoplasmic sodium uptake and potassium loss, and mitochondrial calcium uptake by direct measurements on individual cells. We also found that mitochondrial calcium uptake was accompanied by increases in the phosphorus content of mitochondria (manuscript in preparation). Since phosphate is not present in the extracellular medium we assume that it originates in another intracellular compartment. Here again X-ray microanalysis provided a means of directly measuring elemental changes in subcellular compartments, which could not be measured with any other existing method.

It is neither possible nor desirable for us to discuss all of the applications of X-ray microanalysis. We refer the reader to existing reviews[1,8,9,10]. This paper is intended only to introduce the technique and suggest its range and limitations. We also stress that technology is rapidly improving; for example, a related method, electron energy loss spectroscopy, may soon provide even lower detection limits for biologically important elements.

Although the physical basis for this technique is relatively simple, its application to biological problems is not always straightforward. We have not touched on such knotty problems as beam-induced changes in the specimen, the choice of appropriate standards, or correction for extraneous X-ray signals. The complexity and expense of the equipment required may be discouraging; however, the availability of such instrumentation is increasing, and we can attest from personal experience that this is an area where collaborative research can be rewarding.

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Letter to the Editor

Efflux used as a fad word?

SIR: When molecules escape or are released from a cell, a vesicle, or an organelle, we may speak simply of their *exodus* or their *exit*. Their *uptake* may also be spoken of as *entry*. In such cases we are making no claim that the indicated direction of net migration is the only one. We would expect that the observed net movement contains at least a small component of movement of the molecules in the opposite direction.

If our methods tell us how much of one or both of the one-way movements occurs, then the terms *influx* and *efflux* becomes appropriate. To use influx and efflux where no claim is intended that a one-way flux has been isolated has to be labeled a sophistry.

In enzyme kinetics we often approximate the forward flux by measuring the initial rate of the catalyzed reaction. If our observations are brief enough, the reverse flux can be neglected. The operational term, initial rate, is used more often than forward flux or influx. The parallelism between enzyme kinetics and transport kinetics is obvious.

Another reason for using the word flux carefully is that to physicists and kineticists it may not mean simply a flow, but rather a rate of flow. In my Shorter Oxford Dictionary, that is the sole technical definition offered for flux. In the equation $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$, the quantity $k_{-1}[ES]$ may be called 'the flux' in the direction of dissociation – a concept too useful to chemistry to be lightly blurred. Therefore authors who speak of the 'efflux rate' when they mean the rate of exodus (even where a large component of recapture of the escaped solute is likely) will jar some of their readers on two counts. I think they may show themselves not at home with transport concepts.

H. N. CHRISTENSEN
The University of Michigan,
Dept of Biological Chemistry,
East Lansing, MI 48824, U.S.A.