Letters

The Warburg Effect: Two Years Later

It has been 2 years since my letter was published in *Science* (18 Sept. 1981, p. 1313) retracting the work that I had published together with Mark Spector and several other colleagues (*1*). Since that time, three questions have been repeatedly put to me: What part of the published work is correct? How did it happen? Can such incidents be prevented?

We cannot duplicate any of the described procedures for the isolation of either the sodium, potassium–dependent adenosinetriphosphatase (Na^+,K^+-ATPase) or the alleged members of a tyrosine phosphorylating protein kinase cascade from Ehrlich ascites tumor (EAT) cells. We can demonstrate, however, the presence of a tyrosine phosphorylating protein kinase that phosphorylates a Na^+,K^+-ATPase preparation from dog kidney. This protein kinase can be extracted from EAT plasma membranes with 1 percent Nonidet P-40 and sedimented at 40 percent ammonium sulfate saturation, as described by Spector.

S. Nakamura is currently purifying this tyrosine phosphorylating protein kinase from EAT cells. The enzyme is labile, and progress is slow. At the current stage of purity both α and β subunits of the Na^+,K^+-ATPase become phosphorylated, and we detect both phosphotyrosine and phosphoserine after acid hydrolysis; very recently the tyrosine phosphorylation kinase has been separated from the serine phosphorylating kinase.

We had reported (*1*) that naturally occurring polypeptides act as either inhibitors or activators of protein kinases present in plasma membranes of EAT cells. These protein kinases phosphorylate casein and are not stimulated by cyclic adenosine 3′,5′-monophosphate. I had performed these experiments myself with materials given to me by Spector many months before we discovered that some of the proteins had been iodinated instead of phosphorylated. We have since verified the presence of both protein kinase inhibitor in brain and an activator in preparations of transforming growth factors that have been purified by exclusion chromatography and high-performance liquid chromatography. However, the stimulated protein kinase activity that is present in the plasma membranes of EAT cells does not phosphorylate tyrosine residues (as reported) but serine residues. We have purified two representatives of this new class of protein kinases (polypeptide-dependent protein kinase), one from EAT (*2*) and one from human placenta (*3*). We have found no known growth factor or hormone that substitutes for either the activating polypeptide or for the brain inhibitor.

I proposed many years ago that the Na^+,K^+ pump in EAT cells operates inefficiently (*4*). This proposition was based entirely on indirect evidence obtained from experiments with intact tumor cells. When Spector first came to my laboratory, I suggested that he should try to purify and reconstitute the Na^+,K^+-ATPase from these cells into artificial liposomes. We could then establish directly whether or not such a pump operates as efficiently as the reconstituted pump from normal tissues. At about that time, J. H. Johnson, a postdoctoral fellow in my laboratory, observed that a chloromethylketone derivative of lactic acid inhibited glycolysis as well as a cyclic AMP–independent protein kinase activity in plasma membranes of EAT cells (*5*). This was the basis on which Spector proposed a link between a protein kinase and the defective Na^+,K^+-ATPase, and he soon presented evidence that phosphorylation renders the pump inefficient. He worked hard until the early morning hours and was technically superb. All his experiments were documented by electrophoretic analyses and autoradiograms showing phosphorylation of the β subunit of the Na^+,K^+-ATPase and of several protein kinases. One of my technicians helped with the work and reproduced many of the autoradiograms with the enzymes prepared by Spector. In December 1980 I asked one of the postdoctoral fellows in my laboratory to repeat the experiment on the phosphorylation of the β subunit with preparations of Na^+,K^+-ATPase from different sources to test for specificity (an experiment that Spector had not performed). Spector provided only the protein kinase preparation. The first experiment was a complete failure. I asked Spector to go over the protocol, and he discovered that his enzyme preparation had not been stored at −80°C as he had specified. He supplied a new sample, and the experiment was spectacularly successful. Seven preparations of Na^+,K^+-ATPase from different sources and with different mobilities of the β subunit were found in the autoradiogram to be labeled primarily in the β subunit with other minor bands that differed in each preparation. We cannot repeat these observations.

I have accepted full responsibility for the confusion created by our publications and retracted the work as soon as we realized that some of the data were questionable. Soon thereafter, my grant budget was cut (specifically stated to be a consequence of the Spector incident).

Could this incident have been prevented by better supervision? I believe that in this case there was more supervision and parallel experimentation than in most research projects performed by graduate students, simply because of the excitement generated by the findings. It has been suggested that Spector was under great pressure. Indeed he was, but the pressure seemed to come from inside, not from outside. I repeatedly urged him to slow down and not to work so hard.

Certainly we should use the utmost vigilance to prevent future incidents of this kind. Not only the reputation of individual scientists but the reputation of the scientific community is at stake. Fortunately, in spite of the rapid growth of publications, incidents of this type are rare. Most of our students are reliable and proud of their accomplishments. They should have opportunities to continue to teach and do research without undue pressure. Perhaps one day society will recognize that they are the best investment we have for the future of mankind.

EFRAIM RACKER
Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

References

1. E. Racker and M. Spector, *Science* **213**, 303 (1981).
2. E. Racker, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 2899 (1983).
3. M. Abdel-Ghany, S. Nakamura, J. Navarro, E. Racker, *Biosci. Rep.* **3**, 275 (1983).
4. E. Racker, *A New Look at Mechanisms in Bioenergetics* (Academic Press, New York, 1976).
5. J. H. Johnson, A. Zimniak, E. Racker, *Biochemistry* **21**, 2984 (1982).