Scott, W. A. and H. K. Mitchell. Alternate forms of cytochrome c in the mi-1 (poky) mutant of Neurospora crassa.

The mi-1 (poky) mutant of Neurospora is characterized by its slow growth, cytoplasmic inheritance of its characteristics, lack of cytochromes a and b, and an abnormal excess of cytochrome c. Because of this abnormal excess of cytochrome c, this system is ideal to study the regulation and synthesis of this protein.

Extraction of cytochrome c with bore (Hardesty 1961 Ph. D. Thesis, California Institute of Technology) ond subsequent purification with $(NH_4)_2SO_4$ and column chromotography on either CM Sephadex 25 or Amberlite CG-50 gives two chromatographic species of cytochrome c. Both iso-cytochromes c* hove a sedimentation coefficient of 1.5, thus ruling out the possibility that one species is a polymer. Peptide maps of the two proteins are nearly identical, but preliminary results with thin layer electrophoresis on silica gel show one or more differences between the tryptic peptides of the two proteins.

Figure 1 illustrates the chromatographic elution profiles of cytochrome c extracted from three different ages of poky. Under the inoculation conditions used, the cultures were at the following stages of growth: 22 hours = pre-log growth; 38 hours = log growth; and 123 hours = post-log growth. As shown, iso-2-cytochrome c is the predominant species in young poky cultures, but at 38 hours of growth both cytochromes c are present in nearly equal concentrations. The ratio of iso-1-cytochrome c to iso-2-cytochrome c increases until iso-1-cytochrome c is the predominant form in the 123-hour culture. Older cultures of wild type also contain only iso-1-cytochrome c.

While the poky cytochrome c undergoes this sequential change, the mutant approaches a more normal phenotype. But the striking fact is, the cytochrome c excess is due to iso-2-cytochrome c. The drop in the cytochrome c level then parallels the apppearance of iso-1-cytochrome c.

To answer the question whether iso-2-cytochrome c is converted to iso-1-cytochrome c or whether iso-1-cytochrome c is synthesized de novo, poky has been pulse labeled with uniformly labeled C^{14}-lysine at different ages. Both cytochromes are labeled when present with approximately the same specific activity. Young poky has been pulsed also with C^{14}-lysine and then

allowed to grow in cold medium. Sampling the culture at different times during subsequent growth indicated that the label stays in the cytochrome c during the change from iso-2-cytochrome c to iso-1-cytochrome c.

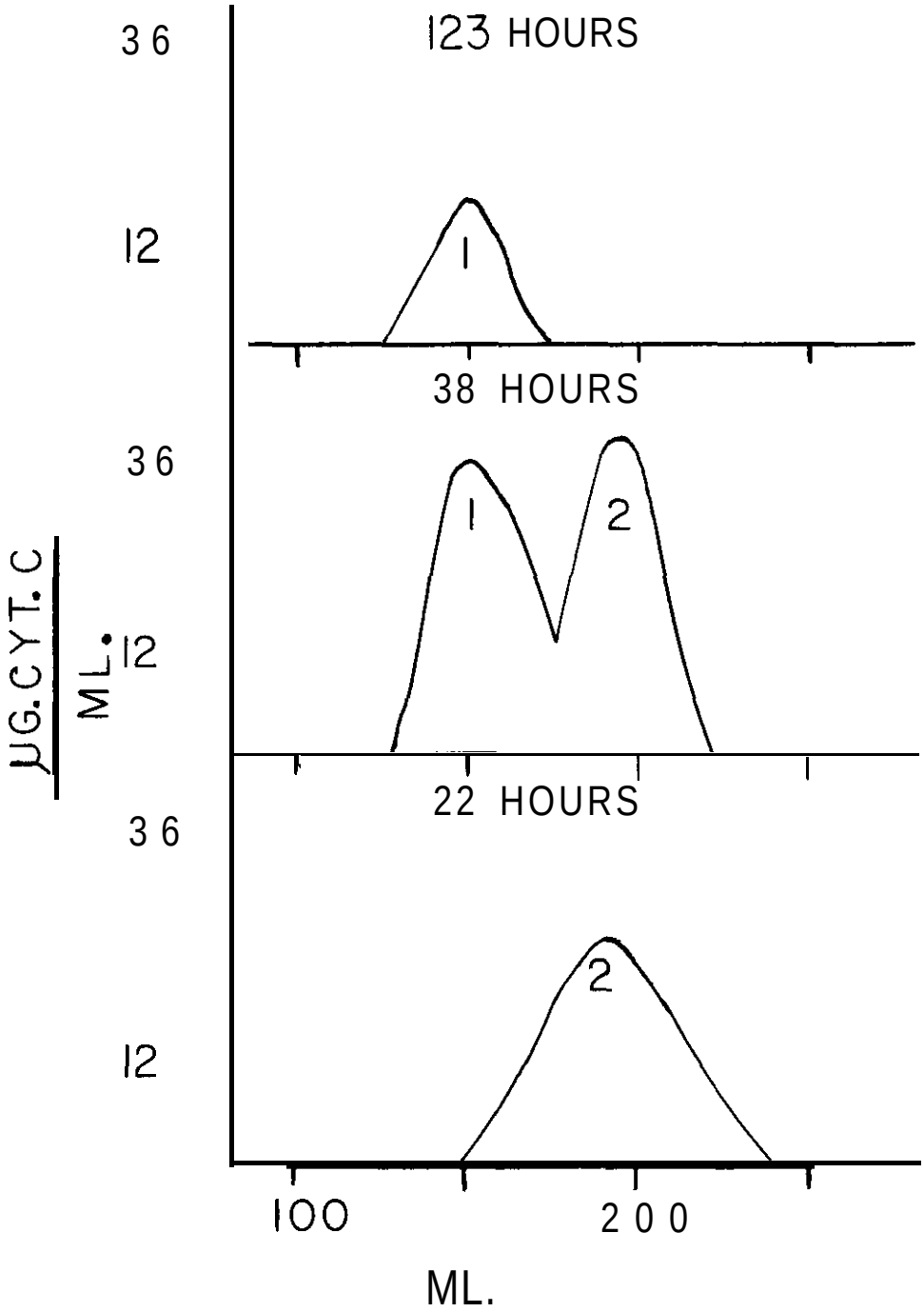

Figure I. Chromatography of cytochrome c isolated from different ages of poky. Partially purified cytochrome c was applied to a I cm. x 50 cm. CM Sephadex 25 column equilibrated with 0.05 M Tris buffer, pH 7.6. The cytochrome c war eluted with a linear gradient prepared by placing 200 ml. of 0.05M Tris buffer, pH 8.6, in the mixing chamber and 200 ml. of I .OM Tris buffer, pH 8.6, in the reservoir.

We conclude that there are two cytochromes c in poky and that they are sequentially produced. Our labeling data further indicates that iso-2-cytochrome c is converted to iso-1-cytochrome c. ▪ ▪ ▪ Biology Division, California Institute of Technology, Pasadena, California.

* The term "iso-cytochromes" was originally used by P. Slonimski, et al. See, for example, A. A. Sels, et al. 1965 Biochim. Biophyr. Acta 95: 486.