**Data Analysis Problem**

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to accompany

*The Cell: A Molecular Approach,* Eighth Edition

Geoffrey M. Cooper

**5.3 Two-Dimensional Fractionation of Proteins Synthesized in a Virus-Infected Cell**

This Data Analysis Problem does not appear in the textbook.

**Source:** Khandjan, E. W., J.-M. Matter, N. Léonard, R. Weil. 1980. Simian virus 40 and polyoma virus stimulate overall cellular RNA and protein synthesis. *Proc. Natl. Acad. Sci. USA* 77: 1476–1480.

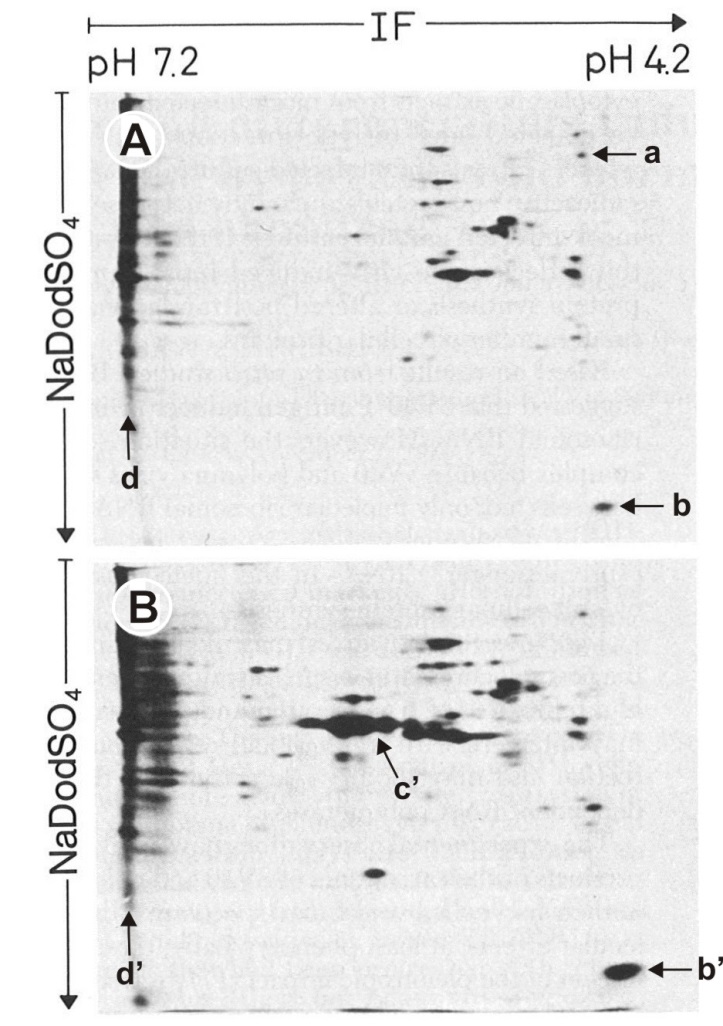
**Corresponding chapter(s) in the textbook:** Chapter 5 (and 10 and 20)

**Review the following terms before working on the problem:** cell cultures, SV40 virus, [35S]methionine labeling, differential centrifugation, two-dimensional electrophoresis, SDS polyacrylamide gel electrophoresis, isoelectric focusing, autoradiography

**Experiment**

Monkey kidney cells were mock-infected (A) or infected with SV40 virus (B) for 46 hours. The cultures were labeled with [35S]methionine for the last hour of infection. Nuclear fractions were prepared, and proteins were extracted from them. Equal amounts of protein were subjected to two-dimensional polyacrylamide gel electrophoresis (1st dimension, horizontal: isoelectric focusing; 2nd dimension, vertical: SDS-polyacrylamide gel electrophoresis). The gels were dried and exposed to an X-ray film. The figure shows the autoradiographs.

**Figure**



Source: Khandjan, E. W., J.-M. Matter, N. Léonard, R. Weil. 1980. Simian virus 40 and polyoma virus stimulate overall cellular RNA and protein synthesis. *Proc. Natl. Acad. Sci. USA* 77: 1476–1480.

**Questions**

1. What was the purpose of the [35S]methionine labeling?

2. What happened to the protein in spot a in the virus-infected cells?

3. What happened to the protein in spot b/b′ in the virus-infected cells?

4. What kind of protein is spot c′?

5. Why did the proteins in spots b′ and c′ migrate differently?

6. Why did the proteins in the long d/d′ spots not move during isoelectric focusing?