

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

AUTORADIOGRAPHY AND POLYACRYLAMIDE GELS:
MEASUREMENT OF RADIOACTIVITY ASSOCIATED
WITH ABSORBANCE TRACINGS¹
AND INDIVIDUAL GEL BANDS¹

*Jerry W. Smith*²
*Terry W. Fenger*¹

Department of Microbiology and Immunology
Louisiana State Univeristy Medical Center
New Orleans, Louisiana

I. INTRODUCTION

In the past few years, high resolution autoradiography has emerged as the single most common method of depicting radiolabeled proteins separated by polyacrylamide gel electrophoresis (PAGE). For the typical publication, photographs are made of autoradiographic film (autoradiograms) containing darkened bands, and used to prepare figures of experimental results (Fenger *et al.*, 1978).

Prior to the use of autoradiography, labeled bands were most often represented graphically as counts per minute (CPM) or disintegrations per minute (DPM) obtained from liquid or crystal scintillation spectrometry of 1-mm gel sections. While eliminating many of the tedious and time-consuming aspects of the sectioning procedures, autoradiography has not, however, been totally free of problems. Perhaps the most important drawback concerns the difficulty one has in assessing the amount of radioactivity represented by the darkened area.

¹Supported by the National Institutes of Health (EY02948, AI10945, AI05744) and the Edward G. Schleider Educational Foundation.

²Present address: Marshall University School of Medicine, Huntington, West Virginia.

An idea of the relative difference among bands can be determined by visual examination of the degree of darkening, as in the case of photographs, or more accurately, by performing desitometric measurements (absorbance tracings) or laborious grain counts (Baserga and Malamud, 1969; Smith and Glorioso, 1977). Since each of these methods, however, is an indirect form of measurement and is affected by the activity of the labeled sample, the type of film, and the times of film exposure, the amount of actual activity remains obscure.

In this report we describe techniques which, while maintaining the advantages of autoradiography, also allow for direct measurement of beta-particle and gamma-ray emissions associated with individual protein bands separated by PAGE.

II. MATERIALS AND METHODS

A. General Procedure

The method generally used for preparing autoradiograms requires that the gels be dry before placing them in contact with photographic film. After fixing and staining with Coomassie brilliant blue, gels were dried in absorbant paper using a slab gel drier and placed in contact with X-ray film. Upon completion of the autoradiogram, a piece of 1-mm graph paper was pasted to the back of the absorbant paper following the dimensions of the individual gel channels outlined by the Coomassie blue staining (Smith and Glorioso, 1977). Each channel was then cut into 1-mm sections and treated for scintillation spectrometry as described below.

B. Preparation of ^{35}S - and ^3H -Labeled Proteins

Hep-2 cells were infected with the Edmonston strain of measles virus and labeled after 6 hr at 37°C with 10 $\mu\text{Ci/ml}$ of either [^{35}S]-L-methionine (1.57 Ci/m mol, Swartz-Mann, Orangeburg, N.Y.) or [^3H]-L-amino acid mixture (New England Nuclear, Boston, Mass.). After 24 hr, labeled cells were harvested and washed extensively with phosphate buffered saline. Cells were treated with NP-40 to solubilize the plasma membrane and the nuclei and other particulate matter were removed by centrifugation. Specific virus antigens in the plasma membrane were precipitated from supernatant fluid by 24 hr of incubation at 4°C with rabbit hyperimmune antisera to measles viurs (Fenger et al., 1978).

C. Polyacrylamide Gel Electrophoresis

The immune precipitate was solubilized by heating at 100°C with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol as previously described (Fenger *et al.*, 1978). Discontinuous SDS polyacrylamide gels were prepared and run by the method of Laemmli (1970). A 3% stacking and a 9% separating gel were employed. Following electrophoresis, gels were fixed by overnight immersion in a solution containing 7% acetic acid and 25% methanol. Polypeptide bands were stained by immersing the gel in a solution containing 0.05% Coomassie brilliant blue, 7% acetic acid and 10% methanol. Destaining was accomplished by frequent changes of the same solution without Coomassie blue.

D. Fluorography, Autoradiography, and Gel Scanning

Fluorography was carried out in gels containing ^3H as described by Bonner and Laskey (1974). Briefly, after destaining, the gel was soaked in Me_2SO , and then in Me_2SO containing 2,5-diphenyloxazole (PPO). The gel was then washed with several changes of H_2O and dried onto absorbant paper using a slab gel drier (Hoeffler, San Francisco, Calif.). Dried gels, including those not treated for fluorography, were placed in direct contact with Kodak single screen film (SB-5) in a Kodak X-ray film holder, covered with aluminum foil, and placed beneath a lead brick in the dark for predetermined periods. Absorbance tracings of the resulting autoradiograms were obtained using a Gilford spectrophotometer equipped with recorder and scanning attachment.

E. Preparation of 1-mm Gel Sections for Scintillation Spectrometry

Following autoradiography, the dried gels were cut into 1-mm sections of individual gel channels (Smith and Glorioso, 1977). Each section was placed in a counting vial and 0.3 ml of Beckman Biosolve (BBS-3). The addition of Biosolve resulted in the dried sections assuming their former thickness, which represented about a ten-fold increase. The addition of 1 ml of NH_4OH also proved acceptable in this regard. After incubation at 37°C until dry, 4 ml of a dioxane-based scintillation cocktail (5 g PPO, 100 g naphthalene/1 dioxane) was added to vials containing sections treated with NH_4OH . Toluene-based scintillator was added to the sections containing Biosolve. The amount of activity present was determined

using a Beckman LS-230 liquid scintillation counter. Counting efficiencies were similar to those obtained with wet (undried) gel slices treated in a comparable manner (Smith and Glorioso, 1977).

III. RESULTS

A. Comparison of Coomassie Blue Profiles, Autoradiograms, Absorbance Tracings, and Counts Obtained by Liquid Scintillation Spectrometry

Figure 1 shows the results of various procedures to differentiate a mixture of labeled (^{35}S) and unlabeled proteins separated by PAGE. Approximately 14 proteins are detected by Coomassie blue staining (Fig. 1A). At least that number appear to be labeled (Fig. 1B), some of which are not visible on the Coomassie blue stain. The radioactivity detectable by scintillation spectrometry of 1-mm sections of the dried gel is shown in Figure 1C and correlates well with the autoradiogram (Fig. 2B) and the absorbance tracings (Fig. 1D) with respect to the number of bands detected. There is disparity, however, in relative heights of the peaks detected in the absorbance tracing, particularly the 3 major peaks.

B. Detection of ^3H -Labeled Proteins

Earlier we found that the activity associated with ^3H -labeled proteins could be extracted and quantitated from dried gel sections (Smith and Glorioso, 1977). The weaker energy ^3H -labeled proteins proved unacceptable for production of autoradiograms, however, probably because of the energy lost through self-absorption (Feinendegen, 1967; Amaldi, 1972). In the present study, we have attempted to use fluorography to circumvent this problem (Bonner and Laskey, 1974). In this procedure, PPO is incorporated into the gel after electrophoresis and destaining (See Methods).

Figure 2 shows the results of separating ^3H -labeled proteins by PAGE with subsequent analysis by staining, autoradiography and liquid scintillation counting of dried sections. As with the results found using the stronger beta-particle emitting nuclide ^{35}S , there was good correlation with all three methods used to detect radioactivity, with liquid scintillation counting providing quantitation. Again, however, there was disparity between absorbance tracing and scintillation counting in the relative heights of the peaks detected.

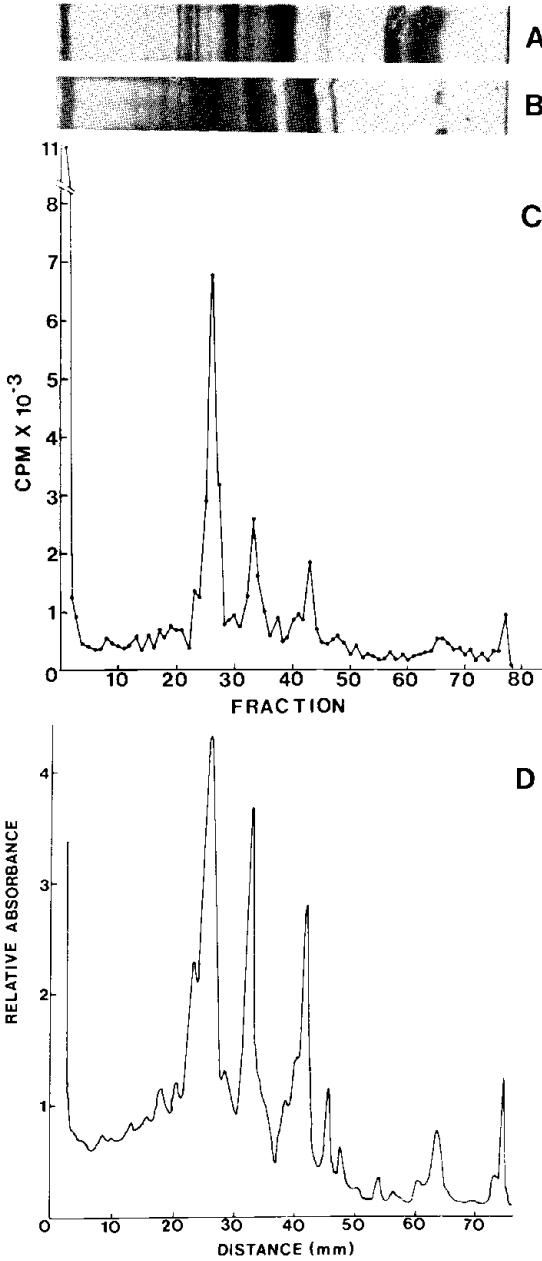


FIGURE 1. PAGE of ³⁵S-labeled proteins of cells infected with measles virus. A. Coomassie blue stain. B. Autoradiogram. C. Radioactivity in 1-mm sections of dried gel. D. Absorbance tracing of autoradiograms.

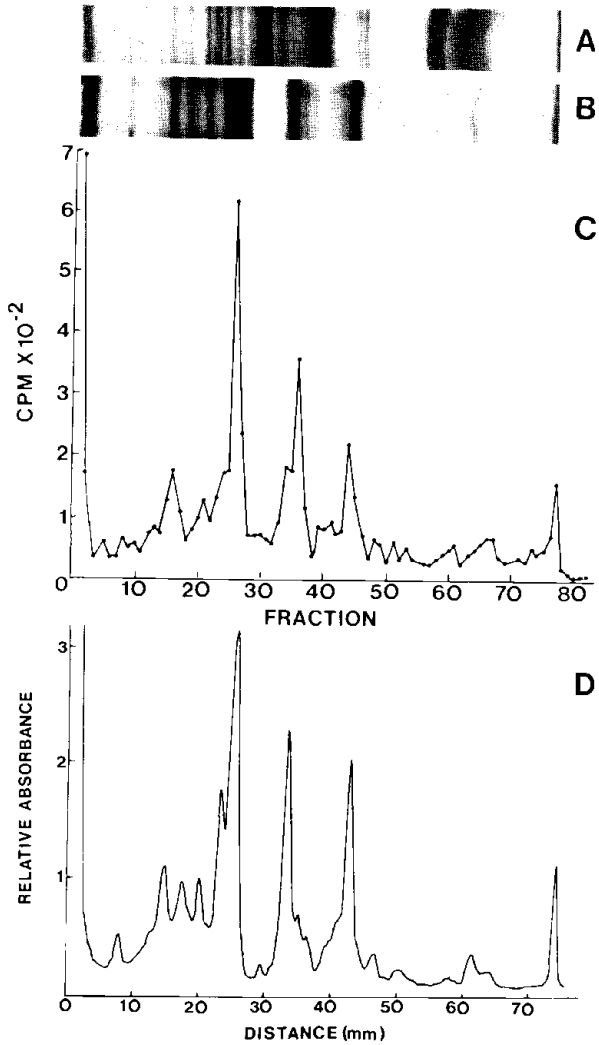


FIGURE 2. PAGE of ^3H -labeled proteins of cells infected with measles virus using fluorography. A. Coomassie blue stain. B. Autoradiogram. C. Radioactivity in 1-mm gel sections. D. Absorbance tracings of autoradiogram.

Attempts were also made to use the special film marketed by LKB (LKB Instruments, Rockville, MD.) for autoradiography of ^3H -macromolecules. We found fluorography to be the more sensitive procedure.

C. Effect of Exposure Period on Detectability of Labeled Proteins by Autoradiography and Absorbance Tracings

One of the problems the reader of an article containing photographs of autoradiograms or absorbance tracings has in evaluating the data presented concerns the actual number of bands or peaks present and the relationship among them. Results shown in Figure 3 indicate the ability to detect labeled proteins and their apparent relationships can be altered by the length of exposure period. After one day of exposure only a few bands can be seen in the photograph or absorbance tracing (Fig. 3C). At 3 days (Fig. 3B), however, all of the major and most of the minor bands can be detected. A longer exposure of 5 days (Fig. 3A) allows the resolution of several minor bands not seen at day 1, and only barely at day 3.

IV. DISCUSSION

In a previous study, we compared counts obtained from gels sectioned without drying, the traditional method, with those obtained from gels dried to produce autoradiograms, and found little loss of activity using the dried gels (Smith and Glorioso, 1977). This finding was true for ^3H , ^{14}C , and the gamma-ray emitting ^{125}I , which very easily could be counted by crystal scintillation spectrometry without rehydration. In this study, we have extended these results to include ^{35}S . In addition, we have presented methods utilizing fluorography by which proteins labeled with weak beta-particle emitters such as ^3H , can be used to produce autoradiograms and then analyzed by liquid scintillation spectrometry.

The counts we obtained from liquid scintillation spectrometry of rehydrated gel sections probably reflect only a small portion of the total activity present (Grower and Bransome, 1970). Release of isotopes from acrylamide gels in general is highly technique dependent and difficult to obtain completely. We have found that counts obtained from rehydrated dried gel sections do not differ significantly from those obtained from wet gel sections treated under the same conditions, indicating that dried sections can be used for quantitation with little loss of activity (Smith and

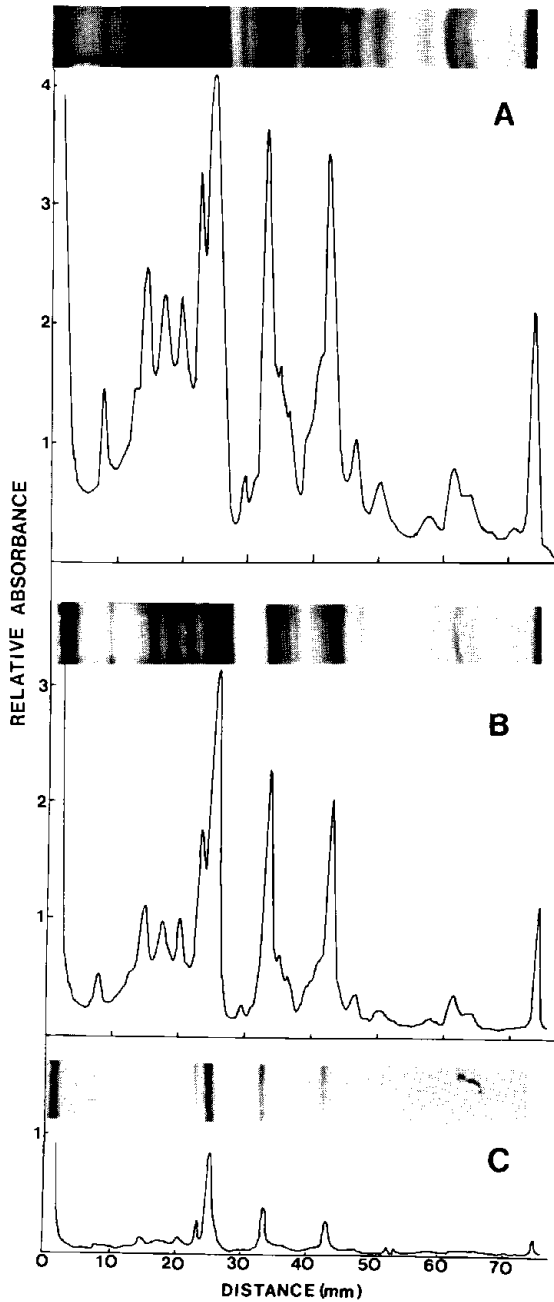


FIGURE 3. Relation of exposure time to development of autoradiograms shown in Figure 2. A. 5 days. B. 3 days. C. 1 day.

Glorioso, 1977). Procedures for highly efficient release of isotope will likely require hydrolysis of the dried gel sections (Grower and Bransome, 1970), or the use of more efficient solubilizers (Gezelius, 1977). Adaptation of such techniques for autoradiographic procedures is being investigated.

Results using both ^3H and ^{35}S showed good correlation among photographs, absorbance tracings, and radioactive counts for detecting labeled bands. Differences were found, however, between scintillation counting and absorbance tracing in the relative heights of the peaks obtained, suggesting that caution should be exercised in interpreting exposed film as a correlate for absolute activity. It is not clear at this point, however, if the problem lies with the exposure technique or with the sectioning procedure. Because of the nature of the sectioning technique, the center of band activity may not be contained in a single 1-mm section, leading to formation of peaks which do not reflect maximum activity.

Exposure time was also found to be very important, as areas containing small amounts of activity could be intensified by long exposure periods, and possibly misrepresented, while in short exposure periods they could be missed altogether. The ability to take the dried gels and determine the radioactive emissions associated with separated bands would clarify such questions.

Additional advantages of using scintillation spectrometry of dried gels in combination with autoradiographs are: 1) dried gels are very adaptable, as they can be used to produce autoradiograms, stored for long periods (depending on the isotope), and reconstituted when needed for measurement of radioactivity; 2) by performing scintillation spectrometry on dried gels used to prepare autoradiograms, it should be possible to distinguish among protein bands labeled with different media, another drawback of autoradiographic techniques.

V. SUMMARY

Autoradiography has become increasingly popular as a method for detecting radiolabeled proteins separated by polyacrylamide gel electrophoresis (PAGE). Bands separated by PAGE are usually illustrated directly as a photograph of the autoradiogram, or as an absorbance tracing. Since the darkening of exposed film is dependent on activity of the labeled sample, the type of film, and the time of exposure, the amount of activity represented by separated bands has

been difficult to determine. Procedures are presented for measuring radioactivity associated with autoradiographic bands, including weak beta-particle emitters such as ^3H . Comparisons of PAGE-separated proteins are made by Coomassie blue staining, autoradiography, absorbance tracing, and scintillation spectrometry of slab gels used to prepare autoradiograms.

REFERENCES

- Amaldi, P. (1972). *Analyt. Biochem.* 50, 439.
- Baserga, R., and Malamud, D. (1969). In "Autoradiography: Techniques and Application", p. 141. Harper and Row, New York.
- Bonner, W.M., and Laskey, R.A. (1974). *Eur. J. Biochem.* 46, 83.
- Feinendegen, L.E. (1967). In "Tritium Labeled Molecules in Biology and Medicine", p. 85. Academic Press, New York.
- Fenger, T.W., Smith, J.W., and Howe, C. (1978). *J. Virol.* 28, 292.
- Gezelius, G. (1977). *Analyt. Biochem.* 80, 627.
- Grower, M.F., and Bransome, E.D., Jr. (1970). In "Current Status of Liquid Scintillation Counting" (E.D. Bransome, Jr., ed.), p. 263. Grune and Stratton, New York.
- Laemmli, V.K. (1970). *Nature(London)* 227, 680.
- Smith, J.W., and Glorioso, J.C. (1977). *Int. J. Appl. Radiat. Isotop.* 28, 693.