# AUTORADIOGRAPHY OF SOLUBLE MATERIALS

O. L. MILLER, JR., G. E. STONE, and D. M. PRESCOTT. From the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Dr. Stone and Dr. Prescott are both presently with the Department of Anatomy, University of Colorado Medical Center, Denver

An autoradiographic method has been developed by which the presence of soluble intracellular materials can be determined for individual cells (1). Our immediate need for the method was to measure the uptake of $H^3$-thymidine into soluble pools during various stages of the cell life cycle, but it should be applicable to the assay of various soluble radioactive materials in a variety of cell types under many experimental conditions. Some results of studies on the uptake of exogenous $H^3$-thymidine by the ciliate *Tetrahymena* and a mammalian tissue culture cell are given to illustrate the method.

## METHOD

### *Preparation of Cells*

For this technique, cells must be prepared for autoradiography by procedures which either prevent loss of soluble material from the cell or limit diffusion of soluble materials to a relatively small area surrounding the cell. Standard freeze-drying techniques are suitable for tissue sections, while both freeze-drying and air-drying can be used for tissue cultures. Most microorganisms can be prepared by rapid air-drying of individual cells or freeze-drying of wet smears on microscope slides. It is essential always that the cells be washed free of exogenous radioactivity before drying or freezing.

To differentiate between soluble and insoluble radioactivity, samples of dried cells are treated in the following ways before emulsion application.

COMPLETE EXTRACTION OF SOLUBLE MATERIAL: Labeled cells mounted on slides are extracted with acid alcohol, trichloroacetic acid, etc., according to the usual procedure. Autoradiographs of such cells demonstrate the amount and location of insoluble (incorporated) radioactivity. Since the soluble materials have been removed, emulsions applied by standard liquid emulsion techniques or by the dry film technique described below give the same results.

NO EXTRACTION OF SOLUBLE MATERIAL: If cells have been prepared in a manner which does not retain soluble materials in the cell but limits the area of diffusion, application of dry emulsion films permits the identification of individual cells from which soluble radioactivity has been derived. The number or types of cells showing soluble *versus* insoluble radioactivity can then be determined by comparison with completely extracted control slides.

If cells have been prepared by a method which

holds the particular soluble material in its *in vivo* location, application of dry emulsion films allows detection of both soluble and insoluble radioactivity within the same cell. If the two components are not coincident in their cellular location, comparison with extracted control slides demonstrates the relative cellular location of the two materials. If the components are coincident in cellular location, the presence of a soluble component can be demonstrated in one of two ways: (*a*) Microextraction prior to emulsion application. In this variation, dried cells are extracted on the slide with small drops of solvent (for example, 3:1 alcohol-acetic acid) applied with a fine bore pipette. The solvent is allowed to evaporate, depositing the extracted material in an area around the cell. The preparation is then covered by the dry film technique. (*b*) Controlled extraction within an emulsion film. In the second variation, water-soluble materials are extracted into a wet gelled film but are retained within a limited area around the cell by subsequent drying of the emulsion.

### *Preparation and Application of Emulsion Films*

NTB3 emulsion (Eastman Kodak Co., Rochester, New York) has been used in these procedures, but other liquid emulsions should be suitable.

DRY FILMS: Emulsion films for dry autoradiography are prepared and applied to specimen slides as follows: 40 to 50 ml of liquid emulsion at 45°C is poured into a 50-ml coplin jar and cooled to near 30°C. An emulsion film is obtained by dipping a wire loop (3.5 to 4 cm diameter) into the emulsion. A loop can be produced easily by twisting size 22 (AWG) nickel-chrome wire around a bottle of the proper size, then attaching the loop to a microscope slide with tape to make a handle (Fig. 1). After dipping the loop, the film is air-dried by placing the slide near the edge of a flat surface so that the loop extends over the edge. The films should be dried for 15 to 30 minutes or until no adherence occurs when touched with a microscope slide.

As shown in Fig. 1, a slide (*S*) with radioactive material located near one end is placed under a dried loop film (*L*) so that the material lies under the center portion of the film. The corners of the specimen end of the slide are then pushed up and slightly over the far side of the loop allowing the end of the slide to push slightly into the emulsion film. The opposite side of the loop is pressed firmly against the middle of the slide so that the emulsion film closely overlies the specimen area of the slide. Moist air (breath) is gently blown (*b*) at the end of the slide until the film begins to adhere at that point. The direction of blowing is then moved slowly toward and over the specimen until the film has adhered to the slide. The slide is pushed through the loop until the non-adhering emulsion is detached.

Tests with dried spots of labeled, water-soluble materials show that the amount of moisture introduced into the film by this procedure does not cause detectable diffusion. In addition, emulsion applied in this manner does not slip or wrinkle during photographic processing. Bubbles may sometimes occur over the specimen, but these are minimized by using the film within a few minutes after drying. Profile sections of dried-down *Tetrahymena* following dry film application show that the film closely adheres to the surface above both nuclear and cytoplasmic portions of the cell.

WET FILMS: A film of emulsion (diluted 1:1 with water) is obtained by dipping a wire loop as previously described. The film is cooled over an ice bath for 1 to 2 minutes and applied to specimen slides similarly cooled. The coated slides are stored for exposure at 5–10°C in boxes containing a drying agent (*e.g.* $CaSO_4$). The amount of water in the cooled, gelled film is sufficient to cause diffusion of water-soluble materials out of the cell, but the extracted materials remain within a relatively small area surrounding the cell.

## EXPERIMENTAL APPLICATION

The uptake of $H^3$-thymidine into soluble cellular pools has been investigated with two cell types, the ciliate *Tetrahymena pyriformis* and a Chinese hamster tissue culture line (CHEF-125-E). Small groups (25–50 cells) of *T. pyriformis* were synchronized by selecting dividers from a log phase culture (2). The degree of synchrony obtained allowed all cells within one group to enter the macronuclear DNA synthesis period with a spread of 10 to 15 minutes or during approximately 1/15 of the total cell generation time of 220 minutes. These groups were then incubated for 15 to 20 minutes in $H^3$-thymidine (10 $\mu$c/ml, specific activity 6.6 c/mM) at known stages of the cell cycle. Using a braking mouth pipette, the cells were washed swiftly and thoroughly through several changes of unlabeled medium (at room tempera-

ture) to remove the exogenous labeled precursor. The cells then were rapidly air-dried on slides by pulling away all excess medium. The actual drying time took 1 or less seconds. Early log phase coverslip cultures of hamster cells were similarly incubated, then washed and frozen-dried or air-dried.

For both cell types, unlabeled control cells in the S period of the cell cycle were introduced into the medium with the experimental cells after the final wash. No incorporation was observed in the control cells even after quite long autoradiographic exposures, indicating that, if these cell types take up $H^3$-thymidine which subsequently can be washed out of the living cell, removal of the unmodified precursor must occur during the brief period of washing in unlabeled medium.

When *Tetrahymena* were incubated during G1 or G2, no radioactivity was detected in either extracted or unextracted cells. Cells incubated during the macronuclear DNA synthesis period and extracted with large volumes of acid-alcohol showed only nuclear (incorporated) label (Fig. 2). When cells similarly incubated but unextracted were covered with wet films, a halo of silver grains appeared over the cytoplasm and around the cell as well as over the nucleus (Fig. 3). When similar S-labeled, unextracted cells were covered with dry films, label again was confined to the nucleus (Fig. 4).

To exclude the possibility that the activity observed after wet film application was the result of endogenous DNase action on DNA, labeled cells were washed and allowed to continue through an additional S period in nonradioactive medium. Samples taken during this period showed that the pools decreased and were entirely absent by the end of the second S period even though the incorporated activity had been reduced, through cell division, only to one-half the amount present following the labeling period.

The results obtained with the tissue culture cells were similar. When these cells were air-dried and covered with dry films, halos of grains appeared (Fig. 5) around all cells which showed heavy nuclear label. The percentage of such labeled cells (210/619) was approximately equal to the percentage of labeled nuclei in extracted control slides (55/159), indicating that the former cells were synthesizing DNA. When air-dried tissue culture cells were covered with wet emulsion films, the halos were dispersed and the soluble radioactivity appeared as

---

FIGURE 1 Diagram of method of dry emulsion film application. Detailed explanation in text.

FIGURE 2 A *Tetrahymena* cell labeled with $H^3$-thymidine during the macronuclear S period and extracted with large volumes of acetic acid-alcohol.

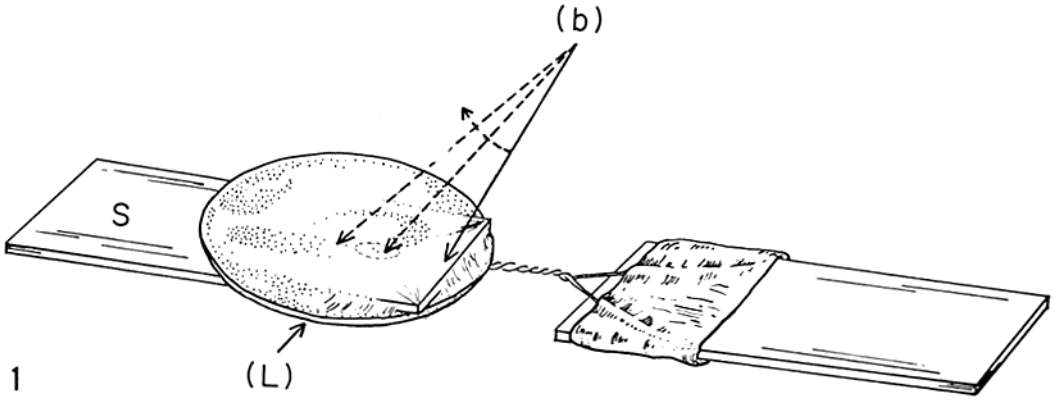
FIGURE 3 An unextracted *Tetrahymena* which was covered with wet, cooled emulsion. The cell exhibits a halo of radioactivity resulting from limited extraction of water-soluble thymidine derivatives.

FIGURE 4 *Tetrahymena* not extracted previous to coating with dry emulsion. The two labeled cells were in early macronuclear S period during incubation while the unlabeled cell was in late G1. In the labeled cells, radioactivity is restricted to the nucleus, showing that the thymidine derivative pool demonstrated by extraction of similar cells (Fig. 3) is located in the nucleus of the cell.

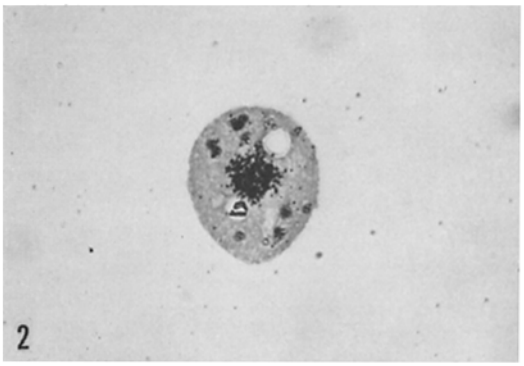
FIGURE 5 Tissue culture cells which were air-dried after incubation with $H^3$-thymidine and then covered with dry emulsion. Two cells are unlabeled while the other two show heavy nuclear label plus a halo of silver grains.

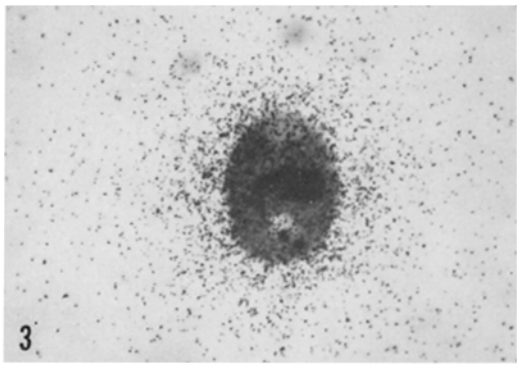
FIGURE 6 Tissue culture cells which were frozen-dried after incubation. The soluble pool which was demonstrated in similar but air-dried cells (Fig. 5) is localized in the nucleus.

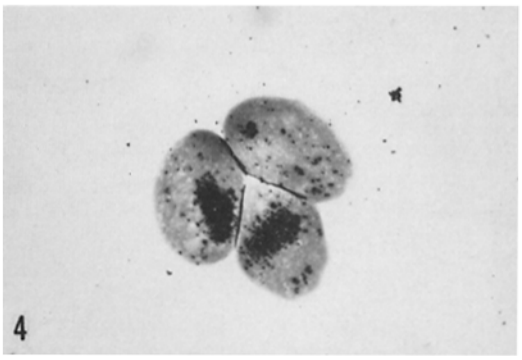
FIGURE 7 Tissue culture cells extracted with acid alcohol.

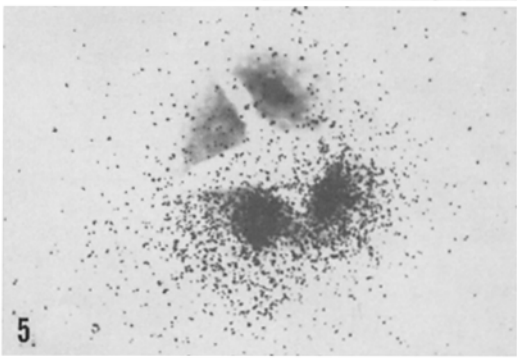


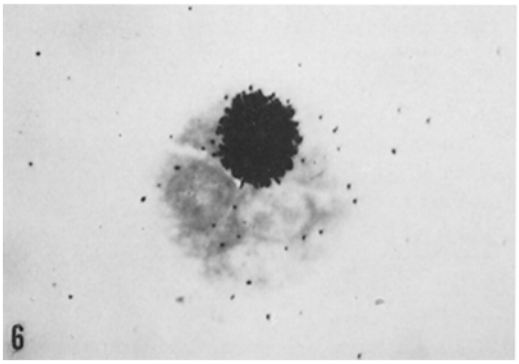
1

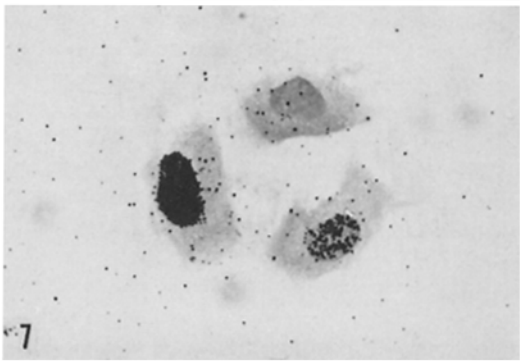

2


3


4


5


6


7

a high homogeneous background. When similar cells were frozen-dried and unextracted, label again was essentially confined to the nucleus (Fig. 6). For comparison, an extracted cell also is shown (Fig. 7).

The application of the described dry autoradiographic technique to two quite different cell types demonstrates that a soluble cellular component which normally is lost during the usual liquid emulsion or wet stripping-film autoradiography can be retained and assigned to individual cells. The results obtained with both cell types also indicate that exogenous thymidine enters into a soluble precursor pool, which can be retained by the cell, only during the S period and demonstrate in a direct manner that this soluble thymidine derivative pool is localized in the nucleus. Two other reports (3, 4) also have provided evidence that this pool is localized mainly in the nucleus.

This research was sponsored by the United States Atomic Energy Commission under contract with the Union Carbide Corporation.

*Received for publication, January 30, 1964.*

## REFERENCES

1. MILLER, O. L., JR., STONE, G. E., and PRESCOTT, D. M., Autoradiography of water-soluble materials, *in* Methods in Cell Physiology, (D. M. Prescott, editor), New York, Academic Press, Inc., 1964, **1**, Chapter 18.
2. STONE, G. E., and CAMERON, I. L., Methods for using *Tetrahymena* in studies of the normal cell cycle, *in* Methods in Cell Physiology, (D. M. Prescott, editor), New York, Academic Press, Inc., 1964, **1**, Chapter 8.
3. BRANTON, D., and JACOBSON, L., Dry, high resolution autoradiography, *Stain Technol.*, 1962, **37**, 239.
4. FEINENDEGEN, L. E., and BOND, V. P., Differential uptake of $H^3$-thymidine into the soluble fraction of single bone marrow cells determined by autoradiography, *Exp. Cell Research*, 1962, **27**, 474.