

# COMBINED AUTORADIOGRAPHY AND ELECTRON MICROSCOPY OF THIN SECTIONS OF INTESTINAL EPITHELIAL CELLS OF THE MOUSE LABELED WITH H<sup>3</sup>-THYMIDINE

JAMES C. HAMPTON and HENRY QUASTLER. From the Division of Experimental Biology, Baylor University College of Medicine, Houston, Texas, and Biology Department, Brookhaven National Laboratory, Upton, Long Island, New York

Recent developments in autoradiography leading to the use of tritiated compounds in the study of biosynthetic processes have provided a basic method for accurate intracellular localization of such labeled substances (1). The high resolution obtained by using such compounds is attributed to the short path of the beta rays emitted by tritium, 50 per cent of which are stopped in less than 1  $\mu$  of tissue. Path distances of such magnitude could probably be obtained with higher energy particles in thin sections covered with thin emulsion. Hence, technics of autoradiography should be seriously considered for more general application in electron microscopy. Limitations in resolution imposed by the emulsion thickness and by the relatively large size of silver grains preclude the possibility of localization of labeled compounds at the molecular or even macromolecular level. But the possibility of observing differences in fine structure between cells or cell components which were, and were not, incorporating labeled compounds at the time of fixation is real and is the main justification for the application of the technics reported here. Autoradiographs of whole liver cell nuclei (2) labeled with cobalt-60, of radioactive dust particles (3), and of sections of tritium-labeled tissues (4) have been studied in the electron microscope. This report presents additional evidence that autoradiographic technics employing H<sup>3</sup>-thymidine can be applied to thin sections for study in the electron microscope.

## MATERIALS AND METHODS

Swiss mice (young adult males of an inbred strain derived from the Walter Reed Hospital pathogen-free stock) were injected intraperitoneally with 750  $\mu$ c. of tritiated thymidine (sp. act. 1.9 C/mM, Schwarz Bio Research Laboratory, Mt. Vernon, New York) dissolved in 1 ml. of saline. About  $\frac{1}{2}$  hour later, the animals were killed, the abdominal cavities were quickly opened, and cold buffered 1 per cent OsO<sub>4</sub> (5) injected into the lumina of segments of ileum. The segments were then removed, cut into small pieces,

and fixed for 1 hour in 1 per cent buffered OsO<sub>4</sub> at about 4°C. The blocks of tissues were embedded in epoxy resin (6), sectioned with a Porter-Blum ultramicrotome, and studied with a Siemens Elmiskop I electron microscope before and after application of the nuclear track emulsion. In an attempt to improve contrast in autographed specimens, the emulsion was enzymatically removed from previously examined grids by floating the grids emulsion side down on the surface of an acid solution of pepsin containing 2 mg./ml. of pepsin at pH 2.0 (7). Sections about 1  $\mu$  thick were prepared for autoradiography and examined with phase contrast optics. Untreated mice of comparable age and the same strain were used as controls in all phases of the work.

The emulsion, Kodak NTA liquid emulsion, was diluted 1:2 with distilled water in a small acid clean vial, heated to 45°C. in a water bath, and applied to the section mounted on the grid with a wire loop in accordance with a method reported by George (3). The sections were stored at 4°C. in light-tight containers for 10 to 14 days, developed in D-19 for 5 minutes, cleared in acid fixer for 5 minutes, and washed in running water for 20 minutes.

## OBSERVATIONS

Fig. 1 illustrates the appearance of three labeled and several unlabeled nuclei as observed in a section exhibiting yellow interference color and coated with emulsion of moderate thickness. Comparison of this illustration with Figs. 2 and 3 will show that the autograph pictured in Fig. 1 was relatively free from contamination by hypo crystals and that the emulsion was quite uniform in thickness. Grains attributed to background can be seen at *G*. Fig. 2 illustrates the appearance of a thicker section than that shown in Fig. 1 but having a somewhat irregular thickness in the emulsion. At first glance, the cell shown in Fig. 2 appears "washboardy" and resembles a familiar defect in sectioning. But close examination will reveal horizontal striations and striations running obliquely from above downward and to the left. These striations are typical of a peculiar pattern

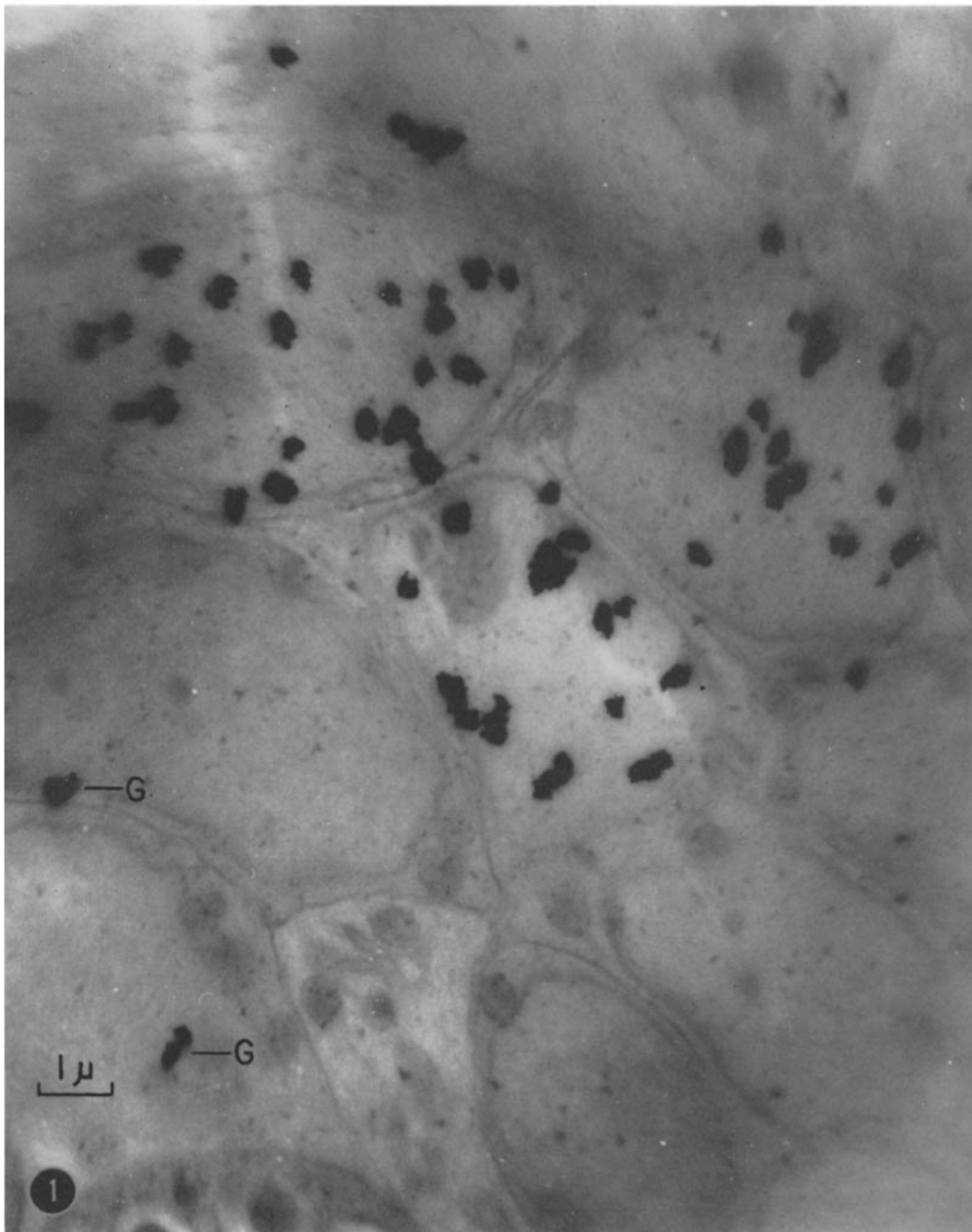


FIGURE 1

A group of autographed cells situated just above the Paneth cells in an intestinal crypt. The groups of black granules represent silver grains over nuclei that had taken up tritiated thymidine. Such granules are about  $0.2$  to  $0.3 \mu$  in size and compare favorably with grain size reported for Kodak NTA emulsion. Grains attributed to background can be seen at *G*.  $\times 10,000$ .

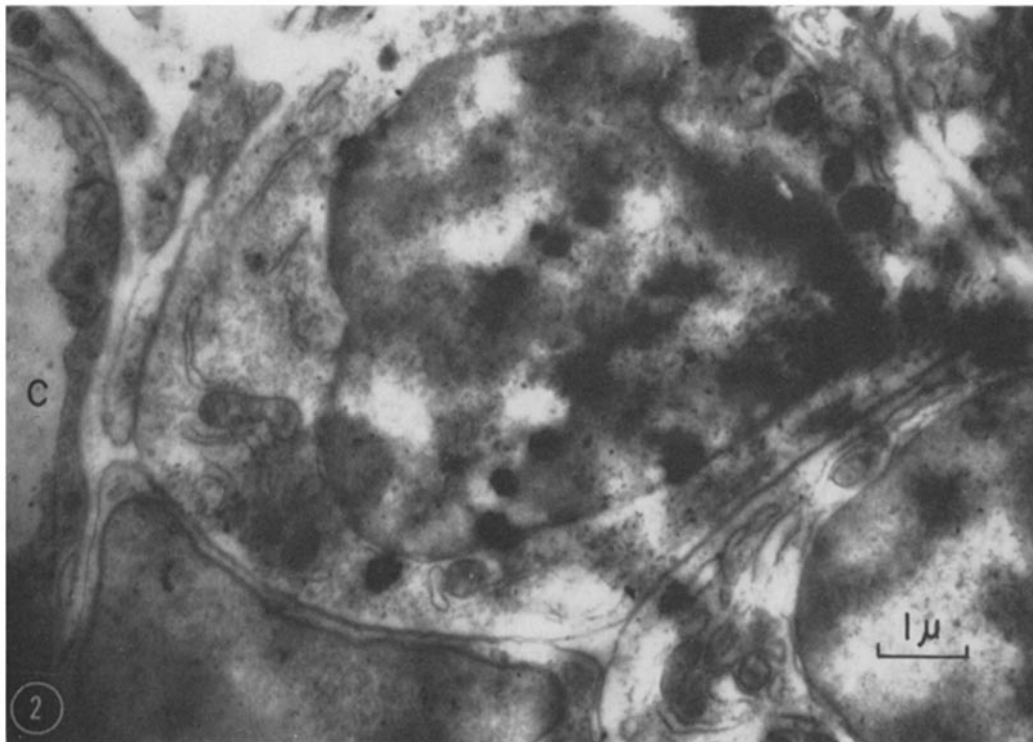


FIGURE 2

A thick section of a single autographed cell in an intestinal crypt. Great variation in emulsion thickness accounts for the patchy appearance of this figure. A portion of a capillary (*C*) can be seen at the left of the picture.  $\times 11,500$ .

of variation in emulsion thickness which was frequently encountered in this work. Fig. 3 shows two labeled nuclei as observed in a thin section covered with a thin layer of emulsion. The heavy crystalline contamination shown in this figure was very common and probably could be traced to hypo that remained after washing. Specimens subjected to pepsin digestion (Fig. 4) appeared to have withstood rather well the rigors of photographic processing, enzymatic digestion, and repeated washings in running water. As might be expected, there was some loss of contrast in the specimen compared to sections examined before the emulsion was applied. The nucleus shown in this figure was one of the few that appeared to retain features of an autoradiograph.

#### DISCUSSION

The work reported here demonstrates that autoradiographic technics can be applied to ultrathin

sections and that such preparations can be studied in the electron microscope. The accompanying illustrations clearly indicate, however, that problems of contrast, contamination, and variations in emulsion thickness are troublesome and difficult to manage.

It was hoped that all three of the above problems could be controlled by the simple expedient of removing the emulsion enzymatically. But, as anticipated, a great reduction in the number of autographed cells in the generative compartment of the intestinal crypt occurred, suggesting that silver grains were removed along with emulsion. Also, other forms of contamination appeared, perhaps fragments of undigested gelatin of about the same size as silver grains, which presented a confusing picture. Other forms of digestion, perhaps partial enzymatic digestion, might prove to be satisfactory. The significant reduction in the number of nuclei labeled with silver grains

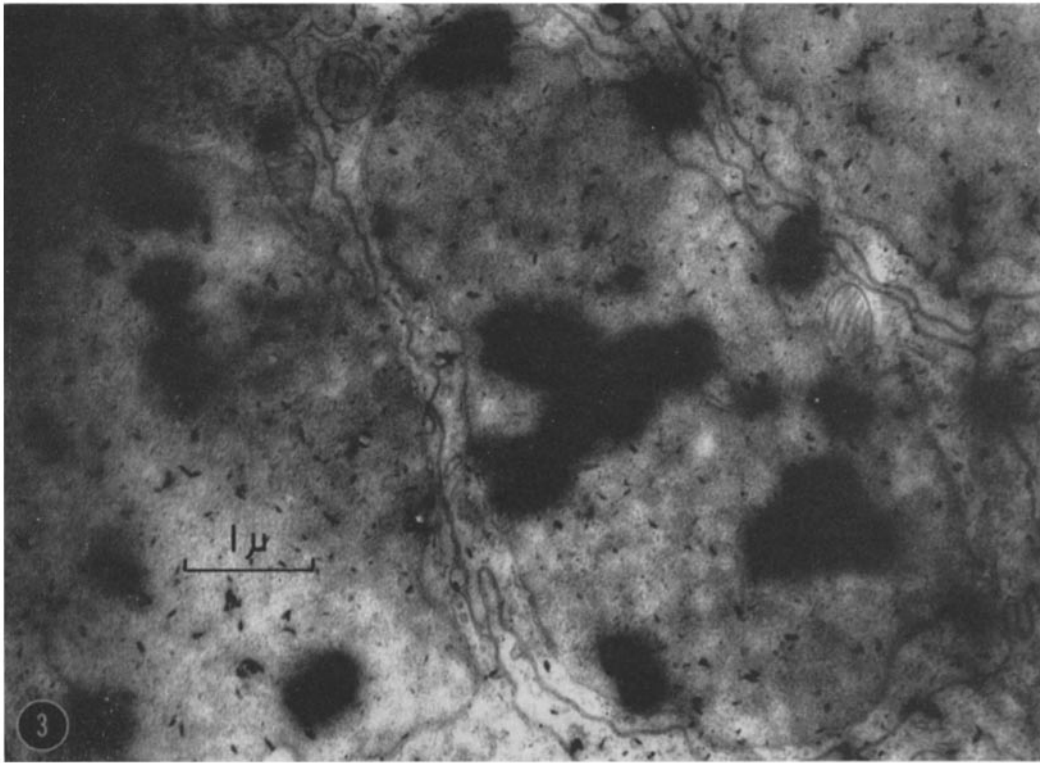


FIGURE 3

A thin section covered with a thin layer of emulsion.  $\times 16,000$ .

following digestion casts serious doubt on the reliability of observations based upon removal of the gelatin. The results thus far obtained have been sufficiently encouraging, however, to warrant continued investigation. Attempts to gain contrast by omitting the supporting carbon film were not very rewarding since the high beam intensity required for sufficient illumination usually destroyed the section.

In spite of the difficulties mentioned above, it is possible to take high resolution electron micrographs of cells, to apply autoradiographic technics, and to find the same cells again in the autographed specimen. Hence, a way is provided whereby cells engaged in biosynthetic processes can be identified and thereby perhaps make available important information on form and function at the subcellular level. The authors do not suggest that ultrastructure at high resolution can be studied directly on the autographed thin section.

#### SUMMARY

Autoradiographic technics have been applied to thin sections of intestinal epithelium labeled with tritiated thymidine and these sections studied in the electron microscope. The observations presented here indicate that the technics of autoradiography and electron microscopy can be combined so that labeled cells can be viewed directly in the electron microscope. The problems encountered were those of low contrast, contamination, and variations in emulsion thickness. Enzymatic digestion of the emulsion was attempted and offers some promise of overcoming all three of the difficulties listed above.

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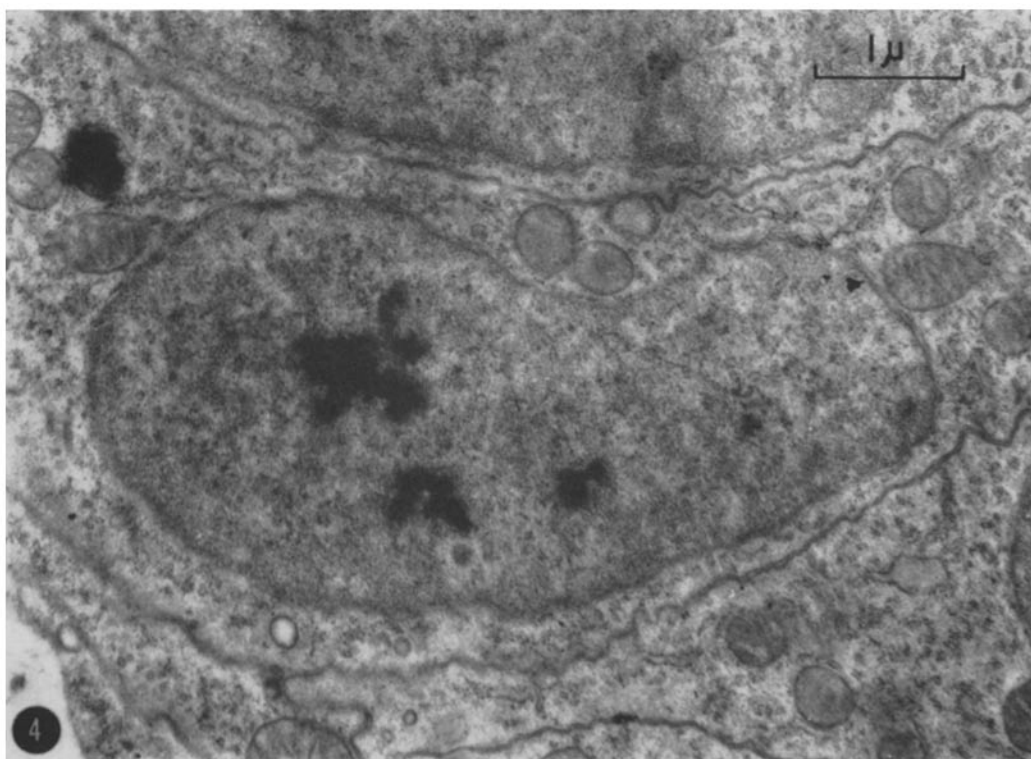


FIGURE 4

A cell observed in an autographed specimen from which the gelatin had been removed by enzymatic digestion. Although the general appearance of a successful autoradiograph has been retained following digestion, there is some doubt whether the particles observed over the nucleus are grains of silver.  $\times 19,500$ .

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#### REFERENCES

1. FITZGERALD, P. J., EIDINOFF, M. L., KNOLL, J. E., and SIMMEL, E. B., *Science*, 1951, 114, 494.
2. LIQUIER-MILWARD, J., *Nature*, 1956, 177, 619.
3. GEORGE, L. A., II, and VOGT, G. S., *Nature*, 1959, 184, 1474.
4. HARFORD, C. G., and HAMLIN, A., *Nature*, 1961, 189, 505.
5. PALADE, G. E., *J. Exp. Med.*, 1952, 95, 285.
6. HAMPTON, J. C., *Texas Rep. Biol. and Med.*, 1960, 18, 602.
7. PEARSE, A. G. E., *Histochemistry, Theoretical and Applied*, Boston, Little, Brown and Company, 2nd edition, 1960.