JOURNAL OF NUCLEAR MEDICINE 5:276-280, 1964

A Staining Technic to Demonstrate Effects of Ionizing Radiation in the Thyroid Gland[1]

James C. Stinson, M.D., James C. Moore, M.D. and Arno W. Sommer, M.D.

Temple, Texas

When histologic examination of individual cells for the effects of radiation is desirable, methods employing photographic emulsions and contact film have some disadvantages. With the oil immersion microscopic technic, for example, simultaneous microscopic study of details in the cells and of particle tracks in the emulsion is almost impossible; therefore, localization of an area of ionizing radiation in a cell is difficult. With this staining technic, the area in which the ionizing energy is or apparently was located can be detected histologically.

MATERIALS

Thyroid glands from human beings and from guinea pigs were used in developing the staining technic. The human material was from thyroidectomized patients. In the patients studied, the dosage of I^{131} ranged from 1.23 to 1.45 microcuries, and the radioiodine uptake at 24 hours ranged from 20 to 67 per cent. The controls, of course, did not receive the radioactive isotope. The guinea pig material consisted of the thyroid gland of a normal animal, selected to serve as a control specimen, and the thyroid glands of guinea pigs which had received 50 microcuries of I^{131}. The treated animals were sacrificed approximately 24 hours after administration of the radioactive isotope. The percentage of uptake of I^{131} in 24 hours was not checked in the guinea pigs.

[1]From the Department of Surgical Pathology and Pathologic Anatomy (Dr. Stinson) and from the Department of Radiology and Radioactive Isotopes (Dr. Moore and Dr. Sommer) of the Scott and White Clinic.

METHOD

The staining technic is simple to perform and special apparatus is not required.

1. A solution of ammoniacal silver nitrate is prepared by dissolving silver nitrate crystals (approximately 0.1 to 0.2 Gm.) in 50 ml of distilled water. Concentrated ammonium hydroxide is added a drop at a time while the solution is stirred. A brown precipitate will form, but this will disappear as additional ammonium hydroxide is added.

2. Sections of tissue (paraffin sections or frozen sections) are immersed in the prepared solution. In this laboratory a 50 ml beaker is used as the container and several slides can be stained simultaneously. The specimens are placed in the beaker with the tissues on the bottom sides of the slides, and they are separated by sections of applicator sticks placed across the rim of the container. This positioning of the tissues allows the precipitates which may form to fall free from the specimens, and the applicator sticks not only separate the individual slides but serve as handles to agitate them in the solution.

3. The beaker containing the staining solution and sections of tissue is placed in a relatively light-free area. (In this laboratory, it is covered with a box or placed in an opaque container with a lid.)

4. About twice daily, the slides should be agitated back and forth to dislodge

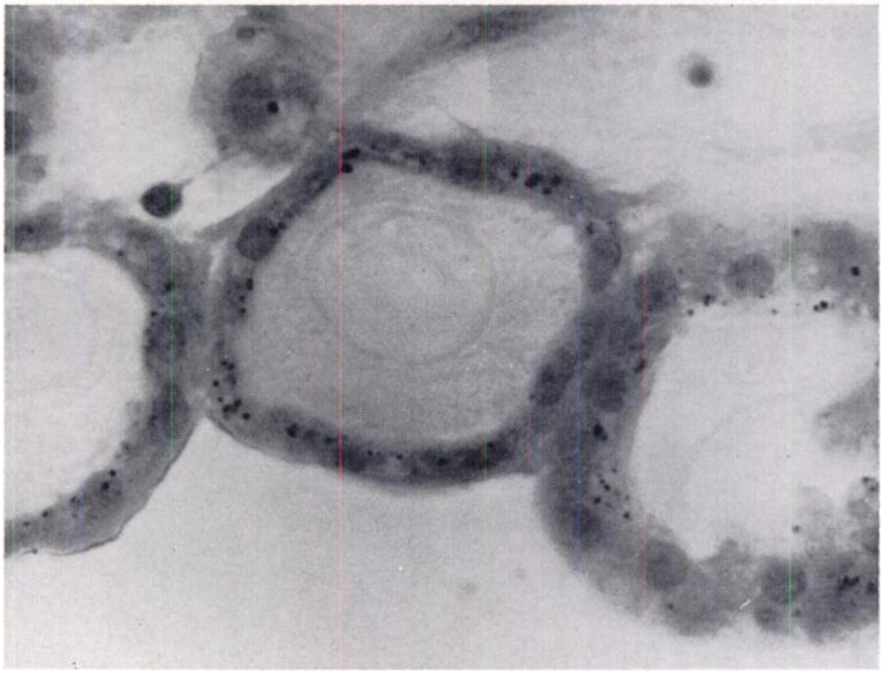

Fig. 1 Section of thyroid gland stained with ammoniacal silver nitrate. The patient had received 1.23 microcuries of I^{131}. (×650)

precipitates and to bring the tissue on the slides into contact with additional solution.

5. After about 24 hours, the tissues may be examined while wet to determine if the desired degree of staining has occurred. A golden brown color is preferable, and additional exposure to the solution may be required to achieve the desired staining.

6. When the desired effect is achieved, the tissues are dehydrated and a cover slip is applied (standard technic).

RESULTS

Sections of thyroid tissue prepared by the staining technic but otherwise unidentified were submitted to pathologists for objective evaluation. In each instance, the irradiated tissue was identified.

In the sections of irradiated thyroid tissue, the cytoplasm of the cells lining some of the acini contained numerous black-stained granules of variable sizes (Figs. 1-4). In the sections of tissue from the control thyroid glands, a rare acinar cell contained an occasional granule; however, difficulty was not encountered in differentiating the irradiated from the nonirradiated tissue.

Particle tracks were not observed in the tissue. If track studies are desirable, emulsion technics must be used in conjunction with the staining method. The staining technic apparently does not permit positive identification of the specific

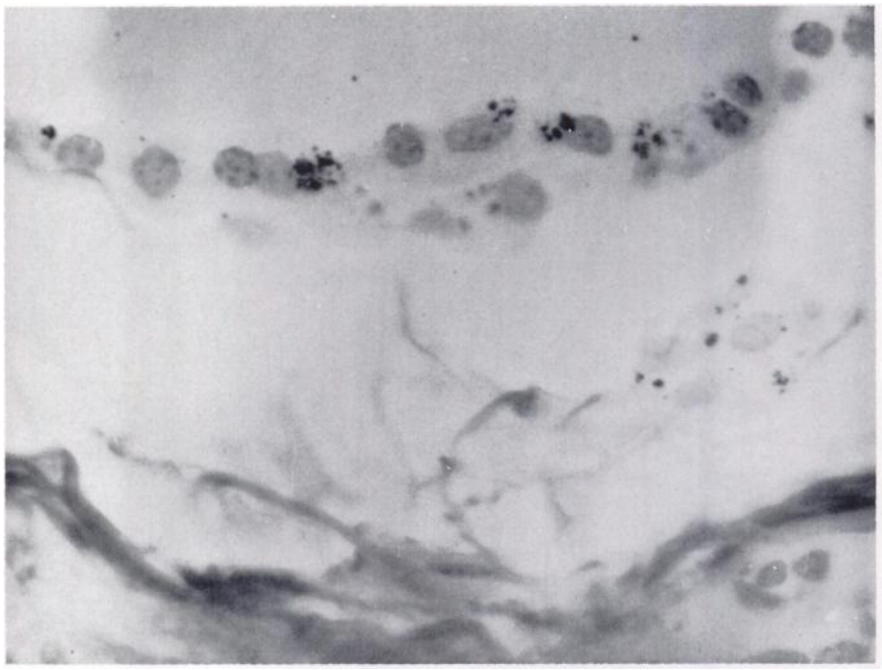

Fig. 2 Section of thyroid gland prepared with the staining technic. The patient had received 1.23 microcuries of I^{131}. (×650)

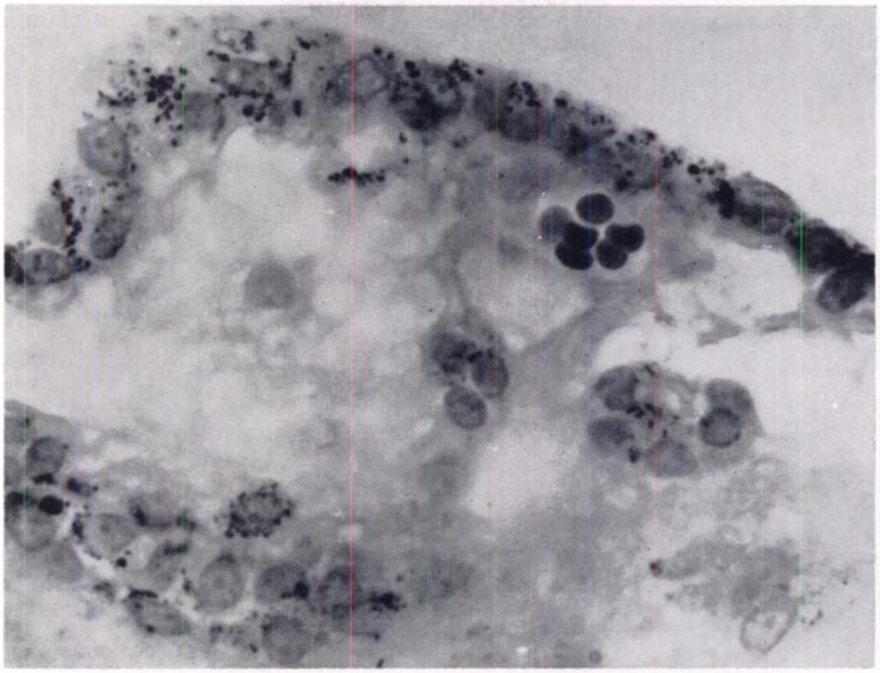

Fig. 3 Section of thyroid gland stained with ammoniacal silver nitrate. The patient had received 1.23 microcuries of I^{131}. (×650)

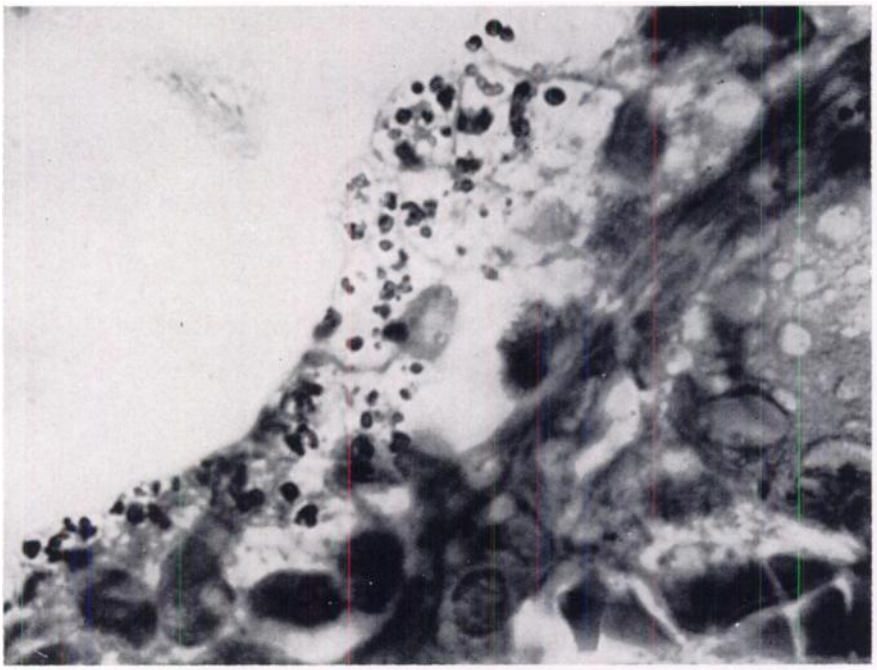

Fig. 4 Section of thyroid gland, treated with ammoniacal silver nitrate, from a guinea pig that had received 50 microcuries of I^{131}. (Oil immersion.) (×1160)

type of ionizing energy acting on the tissue, and the actual presence of radioactivity in the tissue cannot be detected.

DISCUSSION

The black granular material observed in the cytoplasm of the acinar cells is presumed to represent areas in which ionizing energy has been or is present. This presumption is based upon the presence of a large number of granules observed in thyroid tissue known to have been exposed to I^{131} during life and conversely upon a sparsity or absence of these granules in tissue in which there was no known exposure to I^{131}. As the control tissues studies were processed during the same period as the treated glands, radioactive material in the atmosphere would not appear to be a problem. The histologic variation suggests a radiation-induced alteration in the chemical composition of the cells. The dark granules in the cytoplasm probably represent areas of differential staining secondary to the ionizing energy rather than to the actual location of the radioactive focus. These changes may represent the "vacuoles" seen in cells of extensively irradiated tissue when hematoxylin and easin stains are used. In any event, the granular areas are well localized in the cells.

Experiments in radiation chemistry have demonstrated that cross linkage may occur quite readily when certain polymers are irradiated in an aqueous solution. Also, it has been shown that the amount of radiation required for "network" or "gel" formation decreases as the concentration of the polymer is reduced in the solution. Possibly, this experimentally produced reaction is analagous to changes in biological systems subjected to ionizing radiation.[1] If this is true, the presence of the black granules in the cytoplasm of acinar cells could be explained at least in part.

In the development of this technic, most of the tissue utilized was from the thyroid gland. Application of the staining technic to blood and other tissues, however, seems to indicate that histologic alterations secondary to radiant energy may be localized in these other tissues.

REFERENCE

1. Bovey, F. A.: The Effects of Ionizing Radiation on Natural and Synthetic High Polymers, New York, Interscience Publishers, 1958; cited by Charlesby, A.: Ionizing Radiation and Organic Chemistry, *Scientific American* **120**:180, 1959.