INTRANUCLEAR INCLUSION BODIES IN CARCINOMA OF THE THYROID GLAND

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Many authors have written about intranuclear inclusion bodies; their presence has usually been linked with known virus infection, and their association with tumors has been less frequently reported. Intranuclear inclusions have been described, however, by Lucké (1) in adenocarcinoma of the frog's kidney. Russell (2), and Wolf and Orton (3) have reported inclusion bodies in nuclei of the cells of various brain tumors. Many of the spongioblastomata contained them, but none were found in the medulloblastomata. Chemical agents are also a possible factor in their causation, as Olitsky and Harford (4) have shown by producing these bodies with aluminum hydroxide and other compounds. Both intranuclear and extranuclear inclusion bodies were reported by Pappenheimer and Maechling (5) in the epithelium of the renal tubules following bismuth administration. Blackman (6), and Finner and Calvery (7) found intranuclear bodies in the livers and kidneys of children dying of lead poisoning.

In the following case, conspicuous intranuclear inclusions were present in the neoplastic cells of a thyroid carcinoma. Unfortunately, because of the limitations of the necropsy permission, no other tissues could be examined.

M. P. 3740: A seventy-two-year-old business man entered the hospital complaining of a mass in the neck and weakness of two weeks' duration. In the preceding six months he had lost 25 pounds in weight and had noticed increasing weakness. For three or four months he had had an unproductive cough. Two weeks before admission a barber shaved him and massaged his face and neck; on the following day his daughter noticed a swelling in the lower anterior part of his neck. From then on he became weaker; he ate little and the mass in his neck increased in size. Thick tenacious mucus in his throat bothered him greatly and he experienced increasing respiratory difficulty. For one week before admission he had edema of the legs, and for three or four days swelling of the arms.

On physical examination the patient was found to be weak, anemic, and somewhat dull mentally. Several purpuric spots discolored the dorsum of each hand. Across the lower anterior portion of the neck was an elongated firm mass 5 cm. in vertical measurement and 9 cm. from side to side. It extended up the right side of the neck for about 10 cm., and was tender in this region. When the patient swallowed, the mass moved upward slightly. The trachea deviated to the left. On the left side, lateral to the principal mass, was a firm nodule 3 cm. in diameter. A similar nodule was palpated on the right side of the neck separate from the larger mass. The lung bases were dull and breath sounds diminished. The heart was slightly enlarged; the sounds were of poor quality and there was a loud systolic murmur over the entire precordium. Edema of the legs extended above the knees; the arms were edematous to the elbows and there was some edema of the anterior chest wall and back.

Laboratory data were as follows: hemoglobin 4.5 gm. (32 per cent); red blood cells 1,900,000; white blood cells 14,000 (polymorphonuclears 88; lymphocytes 12); serum protein 5.2 per cent; serum albumin 1.9 per cent; serum globulin 3.3 per cent.

Shortly after admission to the hospital the patient had a severe coughing spell and went into shock. His blood pressure rose slowly after a transfusion and eschatin. On the day before death the blood pressure again fell and he died on his fifth hospital day.
Post-mortem examination was limited to the masses in the neck. The large one lay beneath the strap muscles, which were firmly bound to it on the right side. When the mass was removed, the left lobe appeared to consist of normal thyroid tissue; the right lobe and isthmus were replaced by soft grayish-white tissue, arranged in irregular small lobules 2 to 5 mm. in diameter. The right lobe and isthmus weighed 110 gm. and measured $10 \times 4 \times 4.5$ cm.; the left lobe was considerably smaller and weighed 30 gm. On section of the tumor
many golden yellow spots 1 or 2 mm. in diameter were found scattered through it, and there were occasional small hemorrhages. Fragments of normal appearing thyroid tissue remained within the tumor mass. Two round, grayish, encapsulated nodules lay in the left lobe of the thyroid, which otherwise appeared normal. One lymph node was removed from each side of the neck; the one from the left side was replaced by solid, soft, grayish tumor tissue and measured 2.5 cm. in diameter. The node from the right was represented by a fragment of tumor tissue attached to a small ruptured cyst.

**Histologic Examination:** The sections were studied with hematoxylin and eosin, Weigert’s elastic tissue, Foot-Bielschowsky, Mallory’s phosphotungstic acid-hematoxylin, Unna-Pappenheim, and Heidenhain’s iron-hematoxylin stains and by Laidlaw’s method for the demonstration of inclusion bodies.

Segregated small groups of normal thyroid follicles lay in a stroma of edematous collagen. Invading the connective tissue and surrounding the thyroid follicles on all sides were broad, rounded sheets of tumor cells. Necrotic material filled the center of some tumor cell masses; in some places necrosis was widespread. Many of the clusters of well preserved tumor cells were infiltrated with polymorphonuclear leukocytes. The lumina of several small veins were filled with carcinoma cells having an abundant lightly basophilic cytoplasm. In some of the cells the cytoplasm was finely reticular, in others granular. Some cells contained stellate cytoplasmic filaments staining pink with eosin. Large vesicular nuclei occupied about half of the cell body and contained a few small aggregates of chromatin. Mitotic figures were frequent. In almost every nucleus were one or more large, circular or ovaloid eosinophilic inclusion bodies. These were very conspicuous; they measured from 2 to 14 micra in diameter and the larger ones occupied one-third of the nuclear volume. In some instances the inclusion body contained one or several rounded vacuoles. Some of the inclusions, especially the larger ones, were surrounded by a narrow unstained halo; others were covered by granular chromatin. Some of the chromatin was in small clumps, resembling nucleoli, and these stained deeply with hematoxylin. In general there was a rather conspicuous ring of chromatin along the nuclear membrane. Some cells had compact dark cytoplasm and in many of these the nuclear inclusion was large and closely approximated to the nuclear membrane. Many of the nuclei contained more than one of these bodies, some bearing as many as six; the majority, however, contained one or two. Some of the tumor cells were multinucleated and in that case each of the nuclei contained one or more inclusions. Frequently the nucleus was septate, each division containing one or more inclusions. In one cell in which the nucleus was undergoing mitosis, three inclusions were found with the Laidlaw stain in the cytoplasm at the sides of the equatorial plate (Fig. 4). This is an interesting observation as it indicates that inclusion-containing cells are still capable of mitotic division.
With Heidenhain's iron-hematoxylin stain the inclusion bodies appeared black; with the Unna-Pappenheim stain red; with the Laidlaw Orange G stain magenta, and with Mallory's phosphotungstic acid-hematoxylin deep blue. None of the normal thyroid cells had eosinophilic masses in their nuclei, nor did any of the interstitial or inflammatory cells. No colloid was being produced by the tumor.

In general, groups of neoplastic cells were surrounded by a delicate reticulum, but occasionally the reticulum outlined individual cells. The lymph nodes were replaced by tumor having the cytologic features described above.

Thirty-eight carcinomata of the thyroid were examined for the presence of inclusion bodies. They were found in 10. In one of these they were very numerous and conspicuous and resembled those described above. In 9 others they were rare and somewhat less distinct and sharply outlined.

**DISCUSSION**

In pointing out the most consistent features of intranuclear inclusion bodies Olitsky and Harford (4) specify: acidophilic staining, halo formation, and margination of chromatin with or without displacement of nucleoli. Judged by these standards the bodies in the case described may be identified as inclusions. Additional characteristics tend to support this interpretation. The various stages in their development from small discrete to larger bodies can be readily followed and parallel those described by Rosenbusch and Lucas (8) for the inclusion bodies associated with salivary gland virus of guinea-pigs. The giant cells with lobulated or septate nuclei as described are also characteristically associated with certain virus infections. The staining reactions are those typical of other known virus inclusion bodies.\(^3\) As-

\(^1\) It is unfortunate that the Feulgen reaction could not be applied since no tissue fixed for the purpose was available.
assuming that these characteristic inclusions indicate the presence of a virus, the question arises as to whether the hypothetical virus bears any causal relationship to the tumor or whether it has been secondarily implanted in the tumor. Obviously a categorical answer is impossible.

In favor of an etiological relation is the known association of viruses with certain animal tumors such as the Shope rabbit fibroma, infectious myxomatosis of rabbits, and the renal adenocarcinoma of frogs. Furthermore, the occurrence of intranuclear inclusions in 10 of 38 thyroid carcinomata examined makes a chance association less likely. On the other hand, Pearce and Rivers (9) have demonstrated that a spontaneous transplantable rabbit tumor may become secondarily infected with virus III and they succeeded in separating the virus from the tumor, with which it stands in no causal relation. Another possible argument in favor of the secondary nature of the hypothetical virus is the acute inflammatory reaction that is present, unrelated to the presence of bacteria or necrosis of the tumor cells. If the virus is regarded as incidental, it must have a special affinity for the tumor cells as distinct from the normal thyroid epithelium, since the latter contained no inclusion bodies.

**Summary**

A carcinoma of the thyroid is described in which conspicuous intranuclear masses were found within the tumor cells. The question of their significance is discussed. Study of 38 other cases of thyroid carcinoma disclosed the presence of intranuclear inclusions in 10.

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**References**