UNUSUAL PEROXIDASE-POSITIVE GRANULES IN THE DEVELOPING RAT SUBMAXILLARY GLAND

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INTRODUCTION

Unusual peroxidase-positive granules exist in a particular cell type in the rat submaxillary gland during the first 2 wk after birth. The granules display remarkable substructure and clearly show cytochemical evidence of endogenous peroxidase activity. The cells containing the peroxidase-positive granules comprise portions of the terminal end pieces, or so-called "terminal tubules" (4), in the developing submaxillary gland. (The terminal tubules are transient structures that eventually will give rise to mature acini [4]).

Along with the cells containing the peroxidase-positive granules, another cell type also exists in the terminal tubules at birth. It contains homogeneous granules which do not stain for peroxidase. It is not until about the fifth day after birth that a third, acinar type cell, is seen. The purpose of this preliminary report is to focus upon a description of the unusual peroxidase-positive granules as they are seen at different stages of submaxillary gland development.

MATERIAL AND METHODS

Submaxillary glands were dissected from 1-, 5-, 7-, 11-, and 14-day-old Sprague-Dawley rats. Tiny pieces of the gland were fixed for 45 min in half-strength paraformaldehyde–glutaraldehyde fixative (5), buffered to pH 7.4 with 0.1 M sodium cacodylate. After transferring to cold 0.2 M sodium cacodylate buffer, pH 7.4, for 12–16 hr, the tissue was either postfixed in 2% OsO_4–*s*–collidine (1) (for normal morphology), or incubated in a 3,3′-diaminobenzidine tetrahydrochloride–containing medium as previously described (15) for the cytochemical demonstration of endogenous peroxidase activity. Following ethanol dehydration, the tissue was embedded in Epon (10). Sections 1–3μ thick were used to evaluate the cytochemical reaction within the gland, and then thin sections were cut on a Porter-Blum MT-II microtome. Some thin sections were stained lightly with lead (13) before being examined in a Siemens Elmiskop I or Philips EM-300 electron microscope.

RESULTS

Peroxidase-positive granules with subtle spherical, or tubular, profiles are seen in some of the terminal tubule cells of rat submaxillary gland as early as the first postnatal day. By the fifth day after birth, obvious tubular structures are evident in the peroxidase-positive granules (Fig. 1). The presence of the dark cytochemical reaction product (indicative of endogenous peroxidase activity) enhances the substructural patterns of these granules by outlining their unstained tubules. Some of the

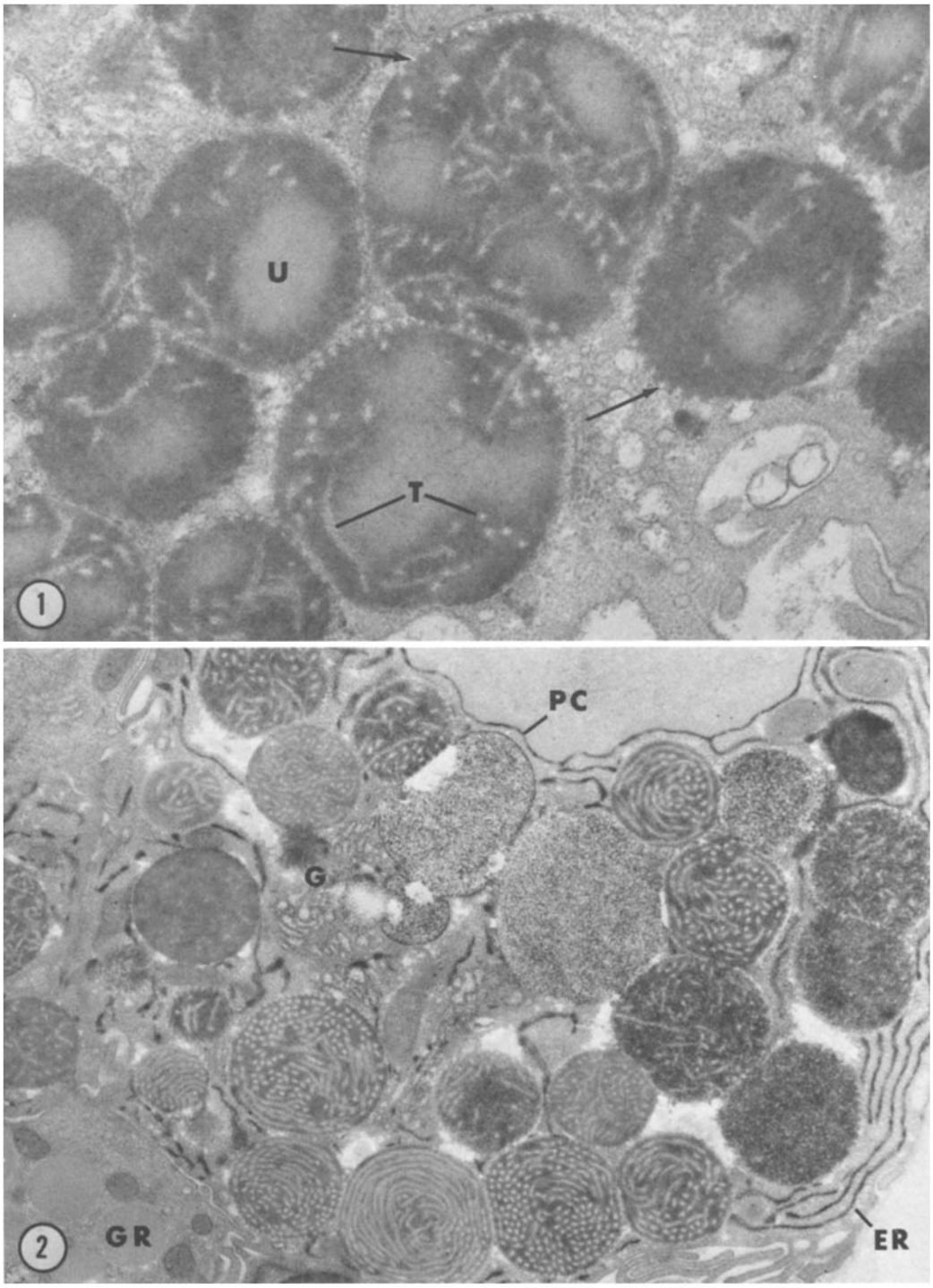

FIGURE 1 Peroxidase-positive granules in terminal tubule cell of the submaxillary gland taken from a 5-day-old rat. Unstained tubules (*T*) penetrate stained portion of the granules, and also rim their periphery so as to give them coglike edges (arrows). Unstained regions (*U*) are also present within the granules. × 38,000

FIGURE 2 A variety of peroxidase-positive granules are evident in this terminal tubule cell from a 7-day-old rat. The perinuclear cisterna (*PC*), endoplasmic reticulum (*ER*), and Golgi apparatus (*G*) also contain the dark reaction product. Compact homogeneous granules (*GR*) in adjacent cell are negative for peroxidase. × 12,800

tubules branch, and occasionally they appear to be interconnected.

The peroxidase-positive granules in terminal tubule cells of 5- and 7-day-old rats have a remarkable appearance. In addition to having their interiors tunnelled out by a number of tubules, many granules also display a periphery that is composed of a highly organized row of unstained tubules (Fig. 1). When these tubules are cut obliquely, the granules exhibit a uniform coglike edge. In sections where the tubules within a granule have been cut longitudinally, a few tubules are seen to extend to the edge of the granule (Figs. 1 and 2), perhaps permitting their interiors to communicate externally with the cytoplasm. Cross-sectioned tubules are occasionally observed to be surrounded by an unstained zone, or halo. Many of the granules also contain one or more regions that do not stain cytochemically for peroxidase (Fig. 1). These unstained pale areas usually have an eccentric location within the granule and they often occupy a large portion of its interior.

Peroxidase-positive granules of various sizes and appearances exist in cells of 7-day-old rats (Fig. 2). These various forms are thought to represent different stages in the maturation of the granules. The perinuclear cisternae, endoplasmic reticulum, and Golgi apparatus contain reaction product, but mitochondria remain unstained. In adjacent cells, compact homogeneous granules negative for peroxidase are evident (Fig. 2). These cells coexist with the cells containing peroxidase-positive granules, but at no time in development have they been observed to contain peroxidase (in contrast to developing acinar cells).

From the seventh through the eleventh postnatal day, there is a decrease in the number of cells containing the peroxidase-positive granules. Moreover, tubular peroxidase-positive granules which appear to be in a state of partial degradation (or transition) are seen. These "degraded" granules do not show as highly an organized tubular pattern as that previously described, nor do they stain as intensely for peroxidase. These altered characteristics make the peroxidase-positive gran-

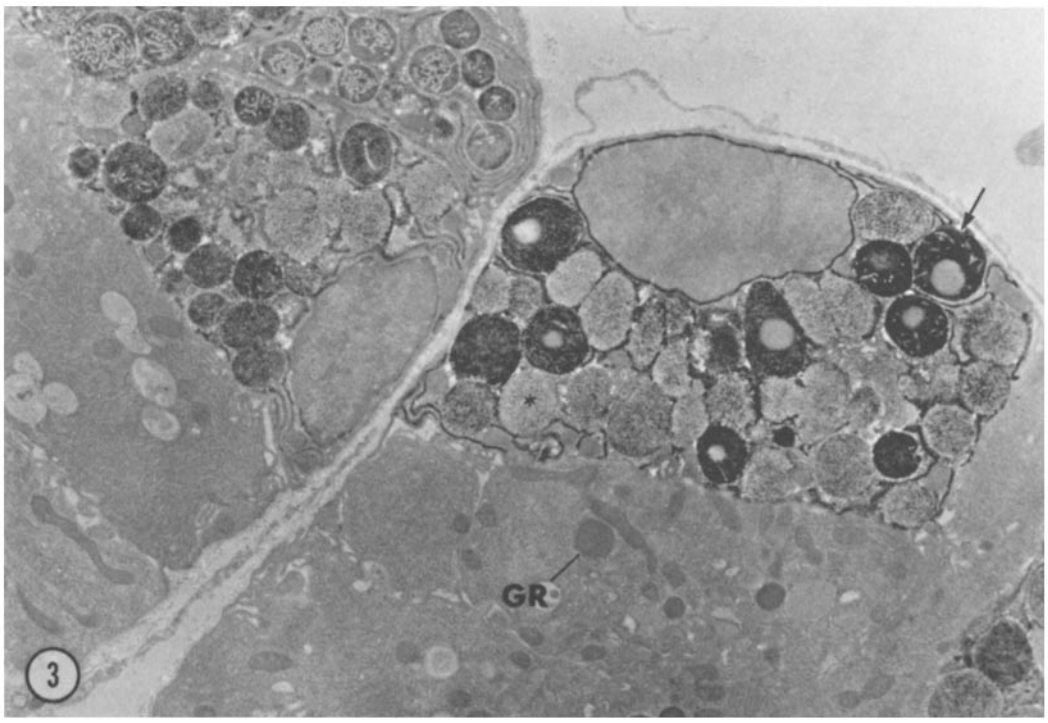

FIGURE 3 An unstained section of submaxillary gland from a 7-day-old rat illustrating portions of two terminal tubules. Peroxidase-positive cells contrast sharply with peroxidase-negative cells. Note cell containing both acinar-like granules (*) and tubular peroxidase-positive granules (arrow). Dense homogeneous peroxidase-negative granules (*GR*) exist in other terminal tubule cells. × 6000

ules similar in appearance to granules sometimes observed in mature acinar cells (15).

Cells containing both tubular peroxidase-positive granules and acinar-like granules are sometimes observed (Fig. 3), but the ubstructural patterns within the acinar-like granules are not typical of most acinar granules seen in the adult gland. Typical acinar-like cells are also present in the differentiating submaxillary gland by the fifth and seventh days after birth, although it is not until the seventh day that they display a positive cytochemical reaction for endogenous peroxidase.

By the 14th day after birth, cells containing peroxidase-positive granules are rare within the gland. Acinar cells clearly staining cytochemically for endogenous peroxidase are now in great abundance. Although the acinar cell granules occasionally exhibit peculiar substructural patterns (which can even appear crystal-like [11]), the patterns differ from the characteristic tubular patterns observed in the peroxidase-positive granules of terminal tubule cells.

DISCUSSION

The unusual peroxidase-positive granules observed in terminal tubule cells of the rat submaxillary gland during the first 2 wk after birth probably represent the "polymorphic" granules described by Dvorak (2) and the granules in duct-type cells reported by Kim et al. (6). A brief look into some of the literature concerned with the postnatal development of the rat submaxillary gland (2, 3, 4, 6, 9, 14) reveals discrepancies regarding an identification of the number of cell types present in the terminal tubules, the nature of their secretory products, and the ultimate destiny of the cells. However, it is generally agreed that the cells of the terminal tubules are precursors of the acinar cells. In agreement with the studies of others (2, 6), the present study confirms that the cells containing peroxidase-positive granules bud off from the terminal tubules. During the fifth through the seventh day after birth, they are the most striking cell type in sections of a gland that has been incubated for the cytochemical demonstration of endogenous peroxidase (Fig. 3).

The cells containing peroxidase-positive granules might function in any of the following ways: (*a*) as specialized cells fulfilling a particular need at a specific time in development, (*b*) as precursor cells that will differentiate into acinar cells, or (*c*) as cells that will give rise to one of the many duct cell types of the adult gland.

Perhaps the specialized cell containing peroxidase-positive granules exists only for the purpose of providing a source of peroxidase in the newborn rat. The enzyme salivary peroxidase has been shown to play an important role in inhibiting the growth of certain bacteria (7, 8, 12). In the adult gland mature acinar cells synthesize peroxidase, but such cells do not exist in the newborn rat. Acinar-like cells are not seen until the fifth day after birth, and initially they do not stain cytochemically for peroxidase. Therefore, it seems that the terminal tubule cells containing peroxidase-positive granules temporarily fulfill the early need for this enzyme. As development proceeds, the cells containing peroxidase-positive granules diminish as the acinar-like cells increase in number.

The cell type containing peroxidase-positive granules has characteristics in common with both acinar cells and certain duct cells of the submaxillary gland. This cell has been termed a duct-type cell because its granules have a density and distinct contour that resemble those of granules seen in cells of the granular duct in adult rat submaxillary gland (6). Moreover, it has been reported that, concomitant with the disappearance of granules in the terminal tubules, secretory granules appear in the granular duct of the developing gland (3, 4, 9). Whether or not the disappearance of terminal tubule granules includes a disappearance of peroxidase-positive granules and/or the compact homogeneous granules remains to be determined. However, cytochemically, this cell is more closely related to the acinar cell in that it synthesizes peroxidase. Its partially degraded granules also appear similar to certain acinar cell granules that contain substructural patterns. The presence of tubular peroxidase-positive granules and acinar-like granules within the same cell favors the theory that the cell is a precursor of the mature acinar cell. However, the initial appearance of acinar-like cells not staining for peroxidase complicates this proposal. It is hoped that a more thorough study using endogenous peroxidase as a cytochemical marker will make it possible to ascertain the fate of the cell containing peroxidase-positive granules.

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