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Letter to the Editor

Salivary gland neoplasms—stem cell histogenesis?

Dear Sir,

In their recent paper Batsakis *et al.* (1989) elaborate on a previously published postulate (Regezi and Batsakis, 1977) that salivary gland neoplasms arise from the population of pluripotential 'reserve' or 'stem' cells present in the intercalated duct system which are responsible both for regeneration within the gland and for the development of metaplasia. Although this concept provides a reasonable rationale to explain the phenotypic diversity of tumours seen in this gland, these cells have never been demonstrated, and substantiation of Batsakis *et al.*'s hypothesis seems to depend almost exclusively on the notion that only cells present in the intercalated duct region of the mature gland have the capacity for self-renewal.

In their paper Batsakis *et al.* place particular weight on a study by Walker and Gobe (1987) as indirect proof of the existence of the putative stem cell and, in our opinion, draw several unwarranted conclusions from that study. In particular they suggest that, following parotid duct ligation and the subsequent disappearance of acinar cells from the gland, 'new acinar cells arise from the terminal or intercalated duct unit'. In fact, what Walker and Gobe demonstrated was that after ligation acinar cells died and were not replaced. The observed increase in the numbers of acinar cells occurred in the *unligated* contralateral gland. These of course could have arisen from pre-existing acinar cells in that gland. Differentiated acinar cells have previously been shown to proliferate after isoproterenol administration (Barka, 1965).

Figure 4 of Batsakis *et al*'s. paper, adapted from Walker and Gobe's, shows the thymidine labelling index for intercalated duct cells, which increased 80-fold following ligation, together with the labelling index in duct-

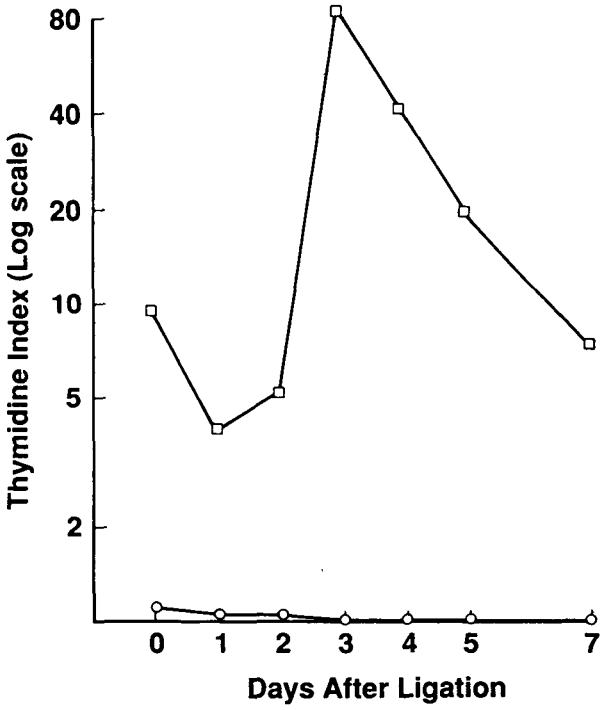

FIG. 4

Thymidine indices of acinar cells (open circles) and intercalated (open squares) duct cells in ligated Sprague-Dawley rat parotid glands (adapted from Walker and Gobe, 1987). After an early fall, counts of labelled nuclei for intercalated ducts increased almost ten-fold by the third post-ligation day. Thymidine indices in acinar cells fell to negligible levels.

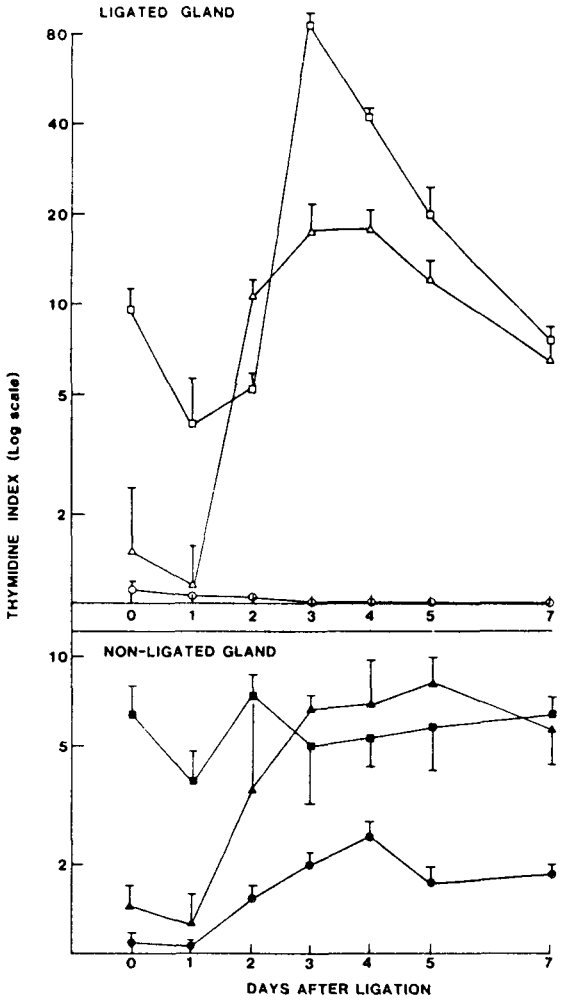

FIG. 15

Thymidine indices of acinar cells (○) and intercalated (□) and striated (△) duct epithelial cells in ligated and contralateral non-ligated parotid glands. Normal control gland values are shown at day zero.

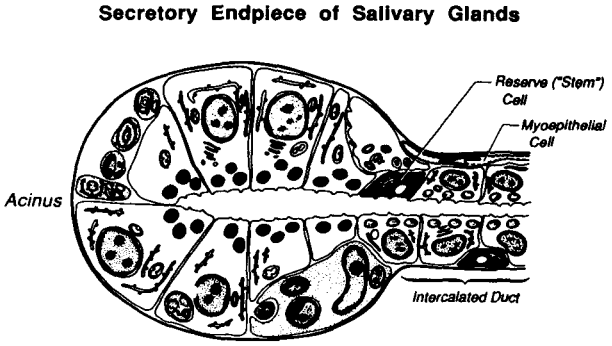

FIG. 3

The reserve or stem cell is at least a tripotent cell, capable of giving rise to duct cells, acinar cells and myoepithelial cells.

ligated glands for acinar cells, which was negligible throughout the study. However, Batsakis *et al.* omitted to include the labelling index for striated duct cells, which increased 20-fold. In addition, data on the unligated gland again revealed a substantial (10-fold) increase in the labelling index in striated ducts, along with an approximately 2-fold increase in acinar cell labelling, but little or no labelling of intercalated duct cells. Any reference to this data was also excluded.

Walker and Gobe's study therefore provides evidence that both striated duct cells (in ligated and unligated glands) and acinar cells (in the unligated contralateral gland) are capable of substantial proliferation. According to the Batsakis model only a cell population resident in the terminal or intercalated duct system has this capacity. The inclusion by Batsakis *et al.* of data from Walker and Gobe's study in support of their hypothesis is therefore potentially misleading.

There is at present very little experimental evidence on which to base histogenetic theories of the development of neoplasms of the salivary gland. It is clear from what evidence is available that, in addition to intercalated duct cells, acinar cells and striated duct cells are capable of replication. We would therefore question the validity of a concept of histogenesis which excludes the possibility of neoplasms may originate from unregulated proliferation of any cell type, fully differentiated or not, present in the normal salivary gland.

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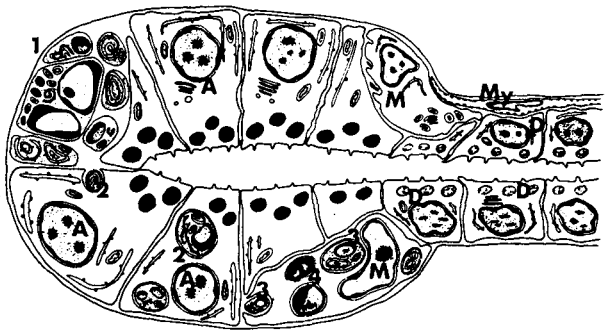

FIG. 17

Diagram illustrating the appearance and fate of apoptotic bodies in parotid gland. (1) Cluster of apoptic bodies from acinar cell in intercellular space. One body contains nuclear fragments with peripheral condensed chromatin. Adjacent acinar cells have closed ranks to reconstitute the luminal surface. (2) Phagocytosis by epithelial cells. (3) Phagocytosis by macrophages. (4) Degeneration and lysosomal digestion. Acinar cells (A), intercalated duct cells (D), intraepithelial macrophages (M), myoepithelial cell (My).

Reply

Dear Sir,

The crux of the argument posed by the writers is in their concluding paragraph and in particular their inability to accept a stem-cell histogenesis, not only for the development of neoplasia but also for normal cell renewal. It is very true that phenotypic characterizations of stem cell populations have proven to be very difficult but their existence in nearly every tissue/organ system in the body is undeniable (Potten and Morris, 1988; Hall, 1989). The epidermis and small intestine are prototypes. In both, tritiated thymidene and radioactively labelled carcinogens have allowed the tagging of the putative stem cell compartment (Potten and Morris, 1988).

It is far easier for me to accept abnormal differentiation from a precursor cell or cells than to believe that a fully differentiated (functionally and cytomorphologically) cell can 'dedifferentiate'. An acinic cell carcinoma, for example, is a neoplastic caricature because of either genetic or epigenetic events that have not allowed normal genomic programming to occur. How else does one explain the solid, mitotically active cellular masses and tubulo-acinar constituents in high-grade acinic cell carcinomas? Surely not by a complete *undoing* of a complicated intracellular mechanism. The writers also ignore the mesenchymal influences on epithelial differentiation by their thesis that fully differentiated structures can give rise to neoplasms (Sharpe and Ferguson, 1988).

The writers confuse maintenance with proliferation and differentiation. It may be that a low level of maintenance is achieved by mitotic activity of fully mature and end-differentiated cells. Repair and replenishment, on the other hand, are through proliferation and differentiation. In salivary tissues both are stem or reserve cell population duties. Under oncogenic stimuli these are aberrant. Under normal conditions, advent of cyto- and functional differentiation is accompanied by a fall in proliferative activity. The reverse is true in the neoplastic state.

The paper by Walker and Gobe (1987) while not dealing with oncogenesis of salivary gland tumors does suggest that it is duct proliferation, in the injured parotid gland, that likely replenishes functional parenchyma. Since our paper deals with tumors of the terminal part of the salivary duct system and not the striated or excretory ducts, we adapted (with credit to Walker and Gobe in the legend) their Figure 15 to show the differences in labelling of the intercalated ducts and acini. The changes in the *non-injured, contralateral* gland, while of interest, do not seem germane to post-injury proliferation in the affiliated gland. Our Figure 3 serves two purposes: (1) to place the stem or reserve cell in the intercalated duct's neck, a hypothetical residency, yet plausible because of our belief it is the precursor to duct and acinar epithelium as well as the myoepithelial cell, (2) it illustrates, as does that of Walker and Gobe's Figure 17 the progressive organelle differentiation from intercalated duct to acini, including secretory products within these cells.

Finally, the writers miss one of the major points of our article, i.e. that in salivary gland neoplasms as in breast neoplasms and proliferative states (Rudland and Barraclough, 1988) it is the myoepithelium that is likely accountable for an ameliorated or low-grade biologic course.

J. G. Batsakis

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Acknowledgments

The Editor is pleased to acknowledge the permission kindly given by the Editor of the Journal of Pathology to reproduce the two figures from the paper by Walker and Gobe.