Growth Pattern of Postnatally Developing Rat Parotid Gland* (33617)

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The salivary glands of the rat are not completely developed at birth and cellular differentiation and organ growth continue for at least 8–10 weeks postnatally (1, 2). Although the differentiation changes have been described in submaxillary (1) and to some extent in parotid (3), little quantitative work has been done to determine the extent to which the separate processes of cellular poliferation and increase in cell size contribute to the course of postnatal growth of these glands. Recent work on neonatal cardiac (4) and skeletal muscle (5) has shown that both cellular proliferation and increase in cell size contribute to the increases in tissue mass which normally occur postnatally. In both of these muscle tissues, cellular proliferation is dominant in the early postnatal period while increases in cell size become the dominant factor underlying the later increases in tissue mass. In muscle, transformation of undifferentiated cells into muscle fibers is not a prominent feature in the postnatal development of these tissues (6); on the other hand, in salivary gland, no acini are present at birth, and transformation of undifferentiated cells into definitive acinar cells is a very prominent aspect of postnatal development (1–3). The present investigation was therefore undertaken to determine the temporal relationship between cellular proliferation and increase in cell size in postnatally maturing gland.

Materials and Methods. Long-Evans rats, ranging in age from 8 days to 6 months, were used in these experiments. Animals were maintained on lab chow and water *ad libitum* after weaning. Unstimulated parotid glands of unoperated rats were removed from animals anesthetized with Nembutal (50 mg/kg i.p.). Glands were rapidly weighed on a torsion balance, and in some cases, whole glands were used for extraction of nucleic acids. In other cases, only a portion of the gland was preserved (Bouin's solution) for histological examination. Histological sections were cut at 6-μ thickness, and stained with haematoxylin and eosin. A Filar eyepiece micrometer was used to measure size of cells and nuclei, and a calibrated field was used to count mitoses. For these measurements (× 430 magnification) 10 cells or nuclei were measured per slide, and 2 slides from each of 7–12 animals were examined. Cell size was estimated in two ways: first, using the eyepiece micrometer, direct measurement of basal width and the height of the cells was made. Since all outlines were indistinct at early ages and these measurements were therefore sometimes difficult to make, a second method was also used. In this method, size of nuclei of the presumptive or definitive acinar cells was measured. It was found that nuclear diameter of these cells was relatively constant (about 6.2 μ) at all ages from 8 through 180 days (Table I). Consequently, the number of nuclei within a given calibrated field could be used to provide a reliable index of cell size. Change in cell density was not a complicating factor in these measurements except at the 8-day stage. Even at that early stage, however, when loose connective tissue was prominent, it was possible to select areas where parenchymal cells approached the same density observed at other ages. For mitotic counts at each age, mitoses per 30 consecutive fields of acinar parenchymal cells for each slide were counted.

Nucleic acids were extracted fom whole glands and amount determined by methods described by Schneider (7), Kochakian *et al.* (8), and Burton (9). The whole gland was homogenized at 0–4° with 0.4 *N* $HClO_4$, and

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the mixture then centrifuged in the cold. The supernatant fluid was discarded and the precipitate was dispersed and washed 3 times with cold $HClO_4$. The precipitate with $HClO_4$ was then heated in a water bath at 90° for 15 min. Total nucleic acids were determined by measurement of optical density at 260 μ. Total DNA was determined using the diphenylamine reaction (9), and total RNA was obtained by subtracting DNA from total nucleic acid (8).

For determination of the water content of gland, tissues were weighed, dried overnight at 105°, and reweighed.

Results. Weight of rat parotid gland showed marked progressive increase with increasing postnatal age, to about 64 days of age; thereafter rate of increase was small (Table I). Histological examination showed that accompanying the changes in weight were marked changes in mitotic rate and size and degree of differentiation of parenchymal cells (Fig. 1). As already described (1, 3), no definitive acini were present at 8 days of age, and loose connective tissue was very prominent. By 16 days connective tissue had been largely replaced by presumptive acinar cells; definitive acini were not seen before 21–23 days of age, and adult-like appearance of the acini was not observed before 5–6 weeks.

Size of acinar (or presumptive acinar) cells increased with time, with the most marked changes exhibited after 23 days of age (Table I). Width of the acinar cells increased from 7 μ at 8 days of age to about 13 μ by 64 days of age, and increased only slightly thereafter. The height of the presumptive or definitive acinar cells also showed a marked increase, from about 7 μ at 8 days to 15 μ at 64 days of age. Since nuclear size was relatively constant (about 6.2 μ) at all ages (8–180 days, Table I), a gradually decreasing number of nuclei per area from 8 days on also was indicative of the fact that cell size was increasing with time. Here, as in the case of individual dimensional measurements, only slight additional changes occurred after 64 days of age (Table I).

TABLE I. Time Course of Postnatal Changes in Body Weight, and Weight, Cell Size, Mitotic Rate, DNA and RNA Content of Rat Parotid Gland.[a]

No. rats	Age (days)	Body wt. (g)	PA wt. (mg)	Size of acinar nuclei (μ)	Cell size (μ)[b] W	× H	No. nuclei /field[c]	No. mitoses /1000 acinar cells	Total DNA (μg/gland)[d]	Total RNA (μg/gland)[d]	RNA/DNA
10	8	13 ± 1	9 ± 1	6.1 ± .1	7.1 ± .1	7 ± .1	36 ± .2	17.8 ± 0.9	32 ± 2	29 ± 4	0.9
8	16	24 ± 0	22 ± 1	5.8 ± .1	8.3 ± .1	9.3 ± .2	35 ± .3	23.8 ± 1.9	158 ± 7	53 ± 12	0.3
9	23	43 ± 8	56 ± 3	6.1 ± .1	9.6 ± .1	10.9 ± .2	28 ± .3	16.6 ± 1.4	407 ± 10	530 ± 35	1.3
12	32	82 ± 2	98 ± 3	5.8 ± .1	11.5 ± .4	12.1 ± .3	19 ± .5	6.4 ± 2.6	493 ± 13	1694 ± 50	3.4
7	49	113 ± 9	143 ± 10	6.1 ± .0	12.0 ± .4	12.9 ± .4	16 ± .3	3.2 ± 1.2	592 ± 27	1576 ± 177	2.7
8	64	150 ± 11	174 ± 28	6.3 ± .1	13.2 ± .6	14.5 ± .4	17 ± .6	0.5 ± 0.4	635 ± 43	1956 ± 89	3.1
10	86	230 ± 16	203 ± 11	6.2 ± .1	12.6 ± .4	12.7 ± .6	15 ± .4	0.7 ± 0.5	865 ± 56	1984 ± 77	2.3
8	180	307 ± 26	238 ± 12	6.3 ± .2	14.1 ± .8	15.3 ± .8	14 ± .4	0.3 ± 0.3	820 ± 28	2799 ± 235	3.4

[a] All values are mean ± SE; cells in each case refer to presumptive (at 8 or 16 days of age) or definitive acinar cells.

[b] W = width of cell at base; H = height of longest dimension, of acinar cells.

[c] Number of nuclei in calibrated field of acinar cells only.

[d] DNA and RNA expressed as total μg of whole parotid (wet weight).

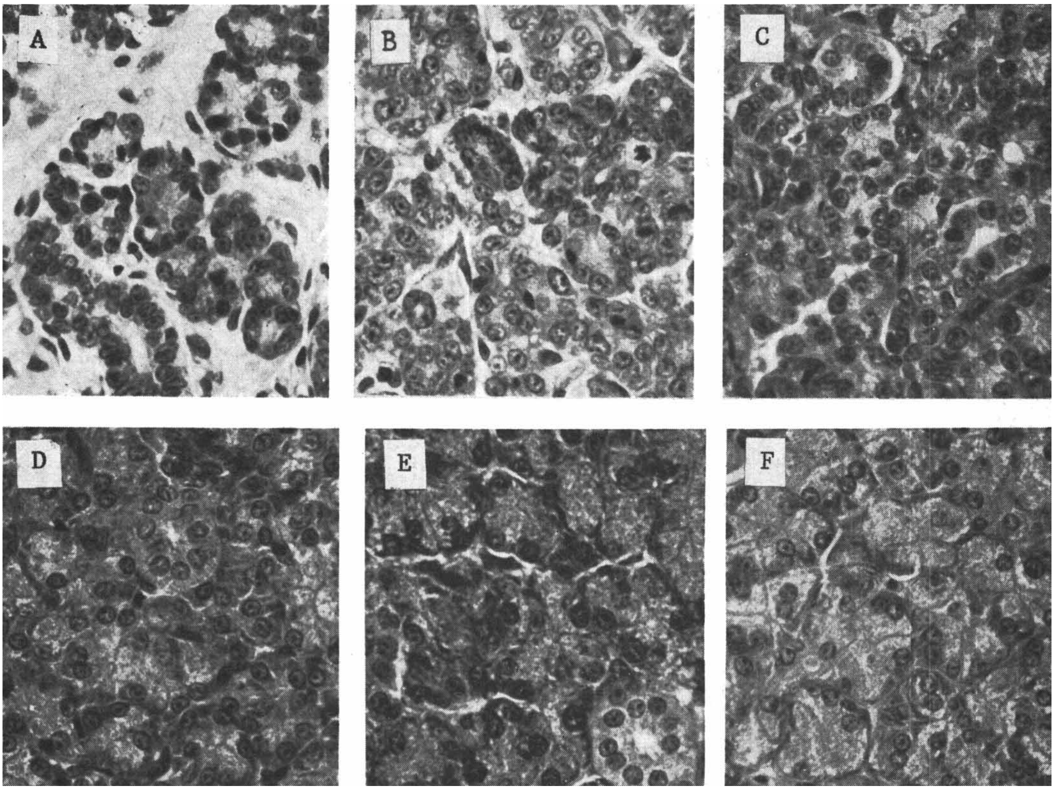

FIG. 1. A. Parotid gland from 8-day-old rat showing ducts, loose connective tissue, and no acini. Mitoses are frequent and cell size small. B, C. 16, 23 days, respectively. Mitoses copious, cell size only somewhat greater than that of cells in A, but connective tissue replaced by cellular elements, many of which are definitive acini. D. (32-day-old) Acinar cell size shows marked increase in D. E. (49 days) Acinar cells continue to increase in size; mitoses very infrequent. F. (180 days of age) Cell size at 64, 86, and 180 days are all similar and only slightly larger than in E; no mitoses evident. ×430.

Cell proliferation of acinar cells or presumptive acinar cells was very significant in the early postnatal periods. Thus, between 8 and 23 days of age, number of mitoses ranged from 17 to 23/1000 acinar cells counted. After 23 days of age, mitotic rate dropped sharply (6/1000 at 32 days). A rate of approximately .5/1000 was generally observed after 49 days of age (Table I). Overall increases in cell number were also reflected in changes in total amount of DNA per gland. A progressive increase in DNA occurred with increasing postnatal age (Table I). The most marked changes in DNA occurred between 8 and 16 days of age (500% increase) and between 16 and 23 days of age (260% increase); thereafter, the increases between intervals were significantly less (between 10 and 30%).

RNA content also increased with increasing postnatal age. The most marked increment was observed between 16 and 23 days of age, and represented an even more significant increase than that observed for DNA. The RNA/DNA ratio, which gives a measure of RNA content/cell, also shows that the RNA/cell increased most markedly between 16 and 23 days.

DNA and RNA content, after reaching relatively stable levels between 49–64 days of age, again increased somewhat after 64 days of age. This change is attributed to manifestation of a new hormonal factor that was not conspicuous before sexual maturity of the animals (60 days of age). Thus, before this time, amounts of DNA and RNA, and gland size were approximately the same in males and females; e.g., at 32 days of age, mean parotid weight of 6 female rats was 104 ± 3 mg and DNA content of the glands 489 ± 17 μg; for 5 male rats, these values were, respectively 105 ± 3 and 486 ± 16; after 60 days, a divergence occurred, and at 180 days, e.g., mean parotid weight for 5 male rats was 275 ± 31 and DNA was 882 ± 35, whereas 5 female rats exhibited mean parotid weights of 202 ± 12 and DNA levels of 760 ± 28.

Water content of the gland decreased from 76% (water/dry ratio = 3.2) at 8 days to about 73% (water/dry ratio = 2.7) at 49–64 days.

Discussion. Rat parotid gland, like cardiac and skeletal muscle (4, 5) shows extensive growth during the first weeks after birth. Parotid mass increases approximately 20-fold during this postnatal period. Cellular proliferation and increases in cell size accompany this marked increase in mass while tissue water changes only to a small extent. As in muscle, cellular proliferation is the dominant event in the first few postnatal weeks. The high mitotic index and concomitant increase in total gland (10) DNA during this period are consistent with this conclusion, especially since the finding of a constant nuclear size at any age at least suggests that polyploidy is not a significant factor in accounting for increased DNA (4, 11–13). In the parotid gland, pronounced differentiation of parenchymal cells also occurs during the early postnatal period. Here again the increasing DNA content suggests that the differentiation from terminal tubule cells into definitive acinar cells involves formation of additional new cells, and not merely replacement of existing cells by new forms of cells (1). Increase in cell size, which has been minimal before 23 days of age, becomes prominent after this time. Measurements, again involving more than one parameter characteristic of the process (i.e., cell size, nuclear counts, and RNA content) (14) provided substantiating data for this conclusion. Furthermore, during this period when cell size is visibly increasing, mitotic rate has dropped sharply, DNA content increases only to a small extent, and DNA concentration drops. These facts as well as examination of RNA/DNA ratios (7) after 32 days of age further support the contention that growth of the gland during this period (after about 3–4 weeks of age) is chiefly attributable to increases in cell size and very little to cellular proliferation.

It is clear then that growth of the rat parotid is effected by cellular proliferation and increases in cell size but that these two events have a variable prominence during the postnatal growth period. Since other neonatal tissue (as heart, and skeletal muscle) exhibit similar biphasic growth (with an early phase of marked cell proliferation accompanied and followed by increases in cell size), a general pattern of postnatal growth appears to evolve, regardless of the extent to which differentiation is involved in the postnatal development.

In other conditions, for instance when growth is induced by partial excision of an organ, by complete excision or other inactivation of an opposite organ (5, 15, 16), or after intense chronic stimulation with chemical agents (17–20), an increase in tissue mass also ensues, but the relative roles of cellular proliferation and increases in cell size apparently do not resemble those generally exhibited by normal postnatal growth. In regenerating adult liver, for example, hyperplasia appears to be the principal factor involved in organ growth (21), while with kidney, at least in the early stages, the enlargement of the remaining organ after unilateral nephrectomy is due chiefly to increased cell size (14, 15). On the other hand, removal of one salivary gland results in hyperplasia in the remaining gland (16); but when salivary gland is stimulated to excessive growth with isoproterenol, cell proliferation and cellular hypertrophy ensue (17–19). Humoral factors, the nature of which remain vague (16), have been implicated to account for the sudden increases in mitotic activity after tissue degeneration or removal (15, 16, 22). Hyperfunction has been implicated in accounting for increased protoplasmic mass after unilateral nephrectomy, cardiac infarction, or isoproterenol-induced increases in heart and salivary gland size (17, 19, 23). It is, however, clear that while increased function may contribute to enlargement induced through hypertrophy, other factors must be involved. For example, pilocarpine has been shown to cause a greater salivary flow than comparable doses of isoproterenol (20); yet the enlargement induced by the latter far exceeds that induced by the former. With unilateral nephrectomy, it is believed that the work load for the remaining kidney is doubled; yet here the remaining kidney does not excrete double its normal load of urea (14). It is thus clear that while postnatal growth exhibits a particular growth pattern in all tissues examined, this pattern is obviously not exclusive for all growth, and the importance or role of regulatory factors (e.g., neural, hormonal, humoral, and function-related) must be assayed.

Summary. The time course of postnatal growth and differentiation of rat parotid has been described, and the mechanisms involved in gland growth examined. An early phase of extensive cellular proliferation, which persists at levels of 17–23/1000 acinar cells through 23 days of age, was seen. This was accompanied by small increases in cell size; after 23 days of age, cell size became the prominent cellular event and mitotic rate decreased markedly. Changes after 64 days of age in gland weight, cell size, state of cellular differentiation, and cell number were not conspicuous. These determinations were based on histological examination and measurement of cell size, nuclear size, and mitotic indices. DNA–RNA content also increased with time postnatally, and both exhibited, like mitotic rate, the sharpest increment between 16–23 days of age. Parallels between the biphasic mechanism of postnatal parotid growth and that of heart and skeletal muscle were examined, and comparison was made to growth patterns exhibited under other conditions of growth.

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