Mitosis Induced in Adult Rat Parotid Following Normal Activity of the Gland[1] (34737)

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In the incompletely developed parotid gland of young rats, when a prior state of functional quiescence is superseded by normal functional activity, DNA increases, and the increase results mainly from increased cell proliferation (rather than decreased cell loss (1). In the adult, however, similar alterations in physiological activity have not been observed to result in any changes in total DNA (2). Therefore, only cell size (and not number) has been thought to be regulated by glandular function in the adult, whereas in the immature rat, both size and number are subject to such regulation (1, 2). The difference in response has been attributed to differences in growth potential of glands in the two age groups: in the adult, mitotic activity is ordinarily very low (3), but is very high in the immature rat (4). However, since under other circumstances (5–7), increases in functional activity can lead to mitotic outbursts in the adult also, cell proliferation might have occurred during the period of transition from low to normal functional status, but because of concurrent cell loss, no appreciable net change in DNA was seen. To uncover a possible link between functional level and gland growth in the adult, the course of change in mitotic activity, as well as DNA, RNA, and gland size, was, in the present work, examined at frequent intervals after stimulation of the quiescent gland by increased masticatory activity, or administration of secretagogues.

Materials and Methods. Long-Evans female rats were maintained on solid laboratory chow and water, *ad libitum,* until 6 months of age. Then, some animals were continued on the solid chow (Ch), while others were fed liquid Metrecal[2] (ME), *ad libitum,* for 7 to 14 days. Metrecal was dispensed from a special container which required only licking for consumption (8). The length of time on ME was varied from maximal times previously used (14 days) to the minimal time actually found to produce maximal effects (about 7 days). Since maximal effects were attained by 7 days, data from all ME-fed animals were considered together. After 7 to 14 days on ME, half of this group was returned to a regimen of solid chow, for a period of 1, 2, 3, 4, or 7 days (ME Ch), while the remainder continued on ME for a corresponding length of time. In some cases, the autonomic blocking agents atropine sulfate (1 mg) or propranolol[3] (Inderal, 2.5 mg) were administered 20 min prior to transfer of the animals to the regimen of solid chow and were given ip three times daily for 2 days. In another set of experiments, rats were maintained for 4 days on water alone. Following this period of fasting, they were fed either solid chow or ME for 2 days. Animals were exsanguinated and sacrificed after anesthetization with pentobarbital, and both parotid glands were removed. One gland was weighed immediately, transferred to ice-cold 0.4 N $HClO_4$, and homogenized for immediate determination of nucleic acids. Total nucleic acid (TNA) was determined using a Hitachi Model 139 spectrophotometer at 260 mμ (9). Total DNA was determined using the Burton modification (10) of the diphenylamine reaction. Total RNA was estimated by the difference between the amounts of DNA and TNA. The second gland was placed in Bouin's fixative

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TABLE I. Time Course of Changes in Cell and Parotid (PA) Size, Mitotic Activity, DNA, and RNA Effected by Introduction of Solid Food to Adult Rats Previously on Liquid Metrecal.[a]

Condition	No. of rats	PA wt (mg)	Mitoses/1000 acinar cells	Acinar cell count (no. of nuclei /unit area)[b]	(μg/gland) DNA[c]	RNA[d]
Con chow[e]	10	196 ± 16[f]	0.02 ± 0.02	16 ± 1.2[f]	647 ± 33	2437 ± 27[f]
ME	18	119 ± 4	0.0 ± 0	25 ± 0.7	644 ± 27	1205 ± 56
ME Ch 1	6	140 ± 8	0.7 ± 0.1[f]	24 ± 1.1	568 ± 40	1558 ± 44[f]
2	15	143 ± 7[f]	22.3 ± 1.9[f]	20 ± 0.3[f]	518 ± 18[f]	1564 ± 48[f]
3	8	177 ± 10[f]	6.0 ± 0.7[f]	17 ± 0.6[f]	627 ± 23	2057 ± 117[f]
4	5	179 ± 10[f]	4.0 ± 1.4[f]	15 ± 1.2[f]	673 ± 59	2188 ± 115[f]
7	6	200 ± 8[f]	0.0 ± 0	15 ± 0.6[f]	689 ± 32	2460 ± 168[f]

[a] Means ± SE.

[b] 60 areas/slide, from regions with acinar cells only, were counted for each animal, and the number of nuclei per area were then counted to obtain estimates of cell size.

[c] Colorimetric determination by diphenylamine reaction.

[d] Estimated as difference between DNA and TNA (determined by spectrophotometric analysis at 260 mμ).

[e] Con chow = rats on solid food only, ME = rats maintained on Metrecal for a minimum of 7, and a maximum of 14 days (plateau in effects reached by 5–7 days); ME Ch 1–7 = rats on ME as above but then placed on solid chow for number of days indicated.

[f] Under each heading, values in the columns indicated with superscript *f* are significantly different from ME ($p < .01$). In addition, mitotic rates for ME Ch 2, 3, and 4 are significantly different ($p < .01$) from those of Con chow and ME Ch 7.

for subsequent histological examination. Tissues were cut at 6 μ and stained with hematoxylin and eosin. Mitotic counts and measurements of nuclear size were made using a calibrated filar micrometer. For each animal, 60 areas, each containing only acinar cells, were counted. Cell size was estimated by determining the number of nuclei per unit area in the histological section, as previously described (4, 9).

Results. Gland size, acinar cell size, and total RNA of parotid glands of adult rats maintained only on liquid diet were 40–50% less than those of rats maintained on solid food (Table I). DNA content and mitotic activity of these glands did not, however, differ from each other (Table I). Mitotic figures were absent or rare in both (0 ± 0 and 0.02 ± 0.02), and DNA was, in each case, about 650 μg/gland (Table I). However, when animals were maintained on Metrecal for 7–14 days and then placed on solid chow for varying periods thereafter (1–7 days of solid food), mitotic activity in the parotid was increased after only 1 day on the chow (0.7 ± 0.1 mitoses/1000 acinar cells, as compared with 0 ± 0 for ME), and reached maximal levels by 2 days (22 ± 1.9 per 1000 cells). Although mitotic activity decreased sharply by 3 days (6 per 1000 cells), it remained conspicuous until 4 days, and only at 7 days were mitoses no longer evident. Significant changes in cell and gland size, and DNA and RNA content ($p < .01$ when compared with ME values) were seen by 2 days (Table I). However, while size and RNA were increased, in most experiments DNA content decreased by about 20% at 2 days (Table I). All parameters increased thereafter and were restored to normal limits within 7 days (*i.e.*, equal to levels seen in rats maintained on solid food) (Table I).

The use of cholinergic or β-adrenergic blocking agents (atropine or Inderal, respectively) modified only some of the usual responses observed after introduction of solid chow to Metrecal-fed rats. After 2 days of chow, cell and gland size increased to approximately the same extent, whether or not the blocking agent was present (Table II). On the other hand, although mitosis was induced by introduction of chow in the presence of

TABLE II. Modification of Chow Induced Changes in Parotid of Metrecal-Fed Rats by Prior Administration of Autonomic Blocking Agents.[a,e]

Condition	No. of rats	PA wt (mg)	Mitoses/1000 acinar cells	Acinar cell count (no. of nuclei /unit area)	(μg/gland) DNA	(μg/gland) RNA
ME	6	112 ± 7	0 ± 0[c]	23 ± 0.5	592 ± 16	1118 ± 104
ME Ch 2[b]	7	161 ± 11[d]	17 ± 4[d]	20 ± 0.3[d]	621 ± 46	1593 ± 212
ME AT Ch 2[b]	7	139 ± 7[d]	4 ± 1[cd]	20 ± 0.5[d]	691 ± 61	1504 ± 216
ME IN Ch 2[b]	7	153 ± 7[d]	2 ± 0.5[cd]	20 ± 0.6[d]	662 ± 18[d]	1300 ± 99

[a] Means ± SE;

[b] To ME rats, nothing (Ch 2), atropine (1 mg) (AT Ch 2), and Inderal (2.5 mg) (IN Ch 2), respectively, were administered, ip, 20 min prior to introduction of solid chow and then continued 3 × daily for the 2 days of chow feeding.

[c] Significantly different from ME Ch 2 ($p < .01$). All other values, in all columns, not different from ME Ch 2 levels ($p > .05$).

[d] Significantly different from ME ($p < .01$).

[e] See Table I for other symbols, and Con chow values.

either atropine or Inderal, the magnitude of the mitotic response in these cases was only 12–24% of that seen with the chow alone (Table II). DNA however, did not change significantly except with Inderal ($p < .01$) and here only a 10% increase was seen when comparison was made with ME rats.

When pilocarpine (PC) was administered 3 times daily for 2 days to ME rats, the number of nuclei per unit area decreased from 28 ± 9 (4 ME rats) to 21 ± 0.5 (4 PC rats); rats fed chow instead showed the same change (21 ± 0.5, 4 rats). Although cell size was reversed to the same extent by either solid chow or PC, increases in gland size and mitotic activity effected by PC were less pronounced than those induced by chow. With PC, a 26% increase in gland size occurred; with Ch, this increase was 45%. Number of mitoses seen after pilocarpine stimulation was 3 ± 0.3 per 1000 acinar cells (4 rats), as compared with 14 ± 2 with chow (4 rats). DNA content did not show any change in either case, and was between 550 and 600 μg/gland.

Mitotic activity in parotid of rats deprived of all food (but not water) for 4 days, was 0 ± 0, but gland size, acinar cell size, and RNA were 20–30% less than levels in chow-fed rats. When the fasted rats were fed with solid food, number of mitoses increased markedly after 2 days of feeding (17 ± 1.5 per 1000 acinar cells). On the other hand, when the fasted rats were refed with liquid food for 2 days, no mitosis was induced (0 ± 0). Cell size, gland weight, DNA, and RNA were not reversed by 2 days of feeding, either with chow or ME (Table III).

Discussion. The present work clearly establishes a relationship between the level of physiological activity and mitosis in adult parotid gland of rat. Previous work has shown such a relationship for the immature animal (1). In the immature parotid, mitotic activity is ordinarily very high (4) but becomes markedly reduced when reflexly mediated activity of the gland is decreased; subsequently the mitotic activity can again be increased by return to normal levels of gland function (1). In the adult gland, mitotic rate is ordinarily very low (3), and a decrease in the functional level below normal does not further reduce mitosis. The present work shows that heightened functional activity results in increased mitosis in the adult just as it does in the immature gland. Thus, regardless of the inherent growth potential of the tissue, sudden increases in functional load lead to bursts of mitosis, although the inherent growth potential may limit the magnitude of the response. It is also clear that unless tissue from the adult gland is examined during the critical 2-day period of transition from low to high physiological activity, mito-

TABLE III. Effects of Refeeding with Liquid and Solid Food on Gland Weight, Mitotic Activity, Cell Size, DNA, and RNA of Parotid of Fasted Rats.[a,e]

Condition	No. of rats	PA wt (mg)	Mitoses/1000 acinar cells	Acinar cell count (no. of nuclei /unit area)	(μg/gland) DNA	(μg/gland) RNA
Fast 4[b]	9	157 ± 4	0 ± 0	22 ± 0.8	727 ± 23	1636 ± 92
Fast 4 ME 2	5	134 ± 8	0 ± 0	25 ± 0.4[c]	647 ± 64	1243 ± 129
Fast 4 Ch 2	10	153 ± 11	17.2 ± 1.5[d]	22 ± 0.6	705 ± 38	1296 ± 163

[a] Means ± SE;

[b] Adult Long-Evans rats maintained on water only for 4 days prior to refeeding with liquid ME for 2 days (Fast 4 ME 2), or solid chow (Fast 4 Ch 2) for 2 days.

[c] Significantly different ($p < .01$) from other values in this column.

[d] Significantly different ($p < .001$) from other values in this column.

[e] See Table I for other symbols.

tic bursts (as well as increases in cell number) may not be observed. Failure to examine the tissue during these critical intervals may also obscure evidence of cell loss. That cell loss occurs is suggested in these experiments by the drop in total DNA, which is observed during the time of maximal mitotic increase (2 days). Since, by 7 days after initiating an increase in functional activity, the drop in total DNA is no longer evident, it appears that, by this time, cell loss has been balanced by the observed cell proliferation. Cycles of cell loss and renewal thus may occur in salivary glands just as in other parts of the gastrointestinal tract. Only by reducing activity of the gland, however, can the regulatory effects of normal gland activity on cell proliferation and cell loss be uncovered.

Functional quiescence was also induced by depriving rats of all food for several days; glands of such rats showed no mitoses. Introduction of solid food to the fasted rats led to induction of mitosis, whereas mitotic activity was not evoked by the introduction of liquid food. Induction of mitosis is thus taken as an indication of increased glandular activity. The present data thus provide additional support for the view that consistency of the diet modifies the level of reflex stimulation of the parotid glands and hence level of glandular activity (8, 11).

The use of autonomic agonists and antagonists has provided further evidence justifying the use of diets of different consistency to modify the level of reflexly-induced glandular activity. Thus, when glandular activity is increased by administration of pilocarpine after a period of functional quiescence, cell number in the adult parotid gland is increased, although the extent of the increase is considerably less than that observed when solid food is introduced. In addition, when Metrecal-fed animals are treated with either cholinergic or β-adrenergic blocking agents (atropine or Inderal, respectively), before introduction of solid food, mitosis, in each case, is only slightly stimulated by the change to solid food.

The use of the autonomic agonists and antagonists also helps to clarify the normal role of the separate autonomic branches in regulating mitosis and cell size. Regulation of these processes is currently not well delineated (7, 12–14), although the autonomic innervation does regulate the overall size of the gland (1, 3, 7, 15–17). The present work indicates that both autonomic branches are involved in reflex regulation of mitosis and cell size, especially since mitosis can still be stimulated, at least to some extent, when either autonomic blocking agent is administered prior to the increased glandular activity associated with the introduction of solid food to ME animals. However, since mitotic activity is much greater when no antagonists are present, both branches must be active for maximal effects on mitosis. Further delineation of the roles of the separate autonom-

ic branches is necessary however, since α as well as β-adrenergic receptors may be involved in the effects.

Summary. When normal function is restored by introduction of a solid food regimen to animals previously maintained on liquid diet (Metrecal) mitotic activity is induced, reaches a maximum after 2 days, (22 $\pm$ 2 per 1000 acinar cells), and remains evident until 7 days. The mitotic burst is partially inhibited in the presence of either atropine or Inderal. A decrease in DNA at 2 days followed by restoration to normal levels at 7 days suggests that, during the course of transition from low to normal functional status of the gland, cell loss must occur but is compensated for by the concurrently occurring cell proliferation. Normal physiological activity thus can act as the stimulus for inducing mitosis in the adult parotid where growth potential is low as well as in the immature parotid where growth potential is high. Glands of fasted rats exhibit similar mitotic bursts following refeeding with solid food, but not when they are refed using liquid food. A role of masticatory activity in this response is thus implied.

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