Influence of Physiological Activity on Mitosis in Immature Rat Parotid Gland[1] (34473)

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Progressive increases in cell number and cell size contribute to the postnatal growth of rat parotid gland (1, 2). However, parotid growth is retarded if, at weaning, animals are maintained on liquid, instead of solid food (2, 3). At all ages examined, DNA content and cell size of parotid glands from animals on liquid diet are less than those of litter mates placed on chow at weaning (2, 3). These effects are attributed to a decrease in neurally-mediated physiological activity of the gland induced by the liquid diet (4), and can be completely reversed if physiological activity is increased by substituting solid food for the liquid diet (3, 4). The increase in DNA content, observed following substitution of liquid by solid food, is of particular interest since this suggested that increased physiological activity is involved in bringing about a net increase in cell number. However, such an increase in cell number could also be the result of diminished cell loss, rather than increased cell proliferation. Therefore, mitotic activity, and the course of its change in relation to DNA content were examined during the 10-day period of chow feeding to animals previously maintained on liquid diet. In addition, the course of change in gland size and cell size and the role of the innervation in regulation of cell size and number were investigated.

Materials and Methods. Long-Evans weanling rats were maintained on a ration of solid chow and water, or liquid Metrecal[2] *ad libitum* Metrecal was dispensed from a special container which required only licking for consumption (4). At weaning, rats were placed on the usual solid chow, (hereafter referred to as "chow regimen," or "chow-fed"), or on liquid-Metrecal diet, ("Metrecal regimen" or "Metrecal-fed"), and were maintained on their respective regimens for 7, 11, or 21 days. In addition, some of the animals maintained on the Metrecal from 21 to 32 days of age, were, at 32 days of age, placed on a diet of solid chow for 1, 2, 3, 5, or 10 days ("Metrecal-chow regimen"). In some groups, under ether anesthesia, complete unilateral postganglionic denervation was performed on 32-day-old rats maintained on Metrecal from 21 to 32 days of age. For varying intervals thereafter (none, 1, 4, 7 days) the Metrecal was resumed, and then chow feeding was substituted for 2 days. Sham controls were also prepared. Rats were anesthetized with 1% pentobarbital, and after exsanguination, both parotid glands were removed. One gland was weighed immediately and transferred to ice-cold 0.4 N $NClO_4$ for immediate nucleic acid determinations. The other gland was placed in Bouin's fixative for histological section. Tissues were cut 6 μ in thickness and stained with hemotoxylin and eosin. Mitotic counts were made using a calibrated eyepiece micrometer, and areas containing only acinar cells were counted. For each animal, 60 areas were counted. Nuclear size was determined with the Filar micrometer. Cell size was estimated

[1] This study was aided in part by Grant DE 02110 (University of Alabama Medical Center) from the National Institutes of Health.

[2] Plain Metrecal was generously supplied by Dr. Harold W. Hermann, Mead-Johnson Research Center, Evansville, Ind.

by counting number of nuclei per calibrated area, since nuclear size was relatively constant (about 6 μ). Total nucleic acids (TNA) were extracted from the gland homogenate as previously described (2), and total DNA was determined using the Burton (5) modification of the diphenylamine reaction.

Results. Parotid glands of rats maintained exclusively on liquid diet (Metrecal) were smaller, had smaller acinar cells, and lower DNA content, than those of litter mates maintained on solid chow from time of weaning (chow regimen). As shown in Fig. 1, this was apparent at all ages examined (27, 32, and 42 days), and the differences between the animals on Metrecal and on chow, for each age, were significant ($p < .01$). Mitotic rates of the two groups were also significantly different from each other ($p < .01$) except at 42 days of age. By 42 days of age, mitotic rate was low in animals on the "chow regimen" (2/1000 acinar cells), and was not appreciably different from that observed in Metrecal-fed rats. Metrecal-fed rats exhibited a low mitotic frequency at all ages examined (Fig. 1).

Introduction of a solid chow diet to animals previously maintained on Metrecal for 11 days after weaning (Metrecal-chow regimen) resulted in marked changes in cell and gland size, DNA content, and mitotic activity. The data in Fig. 1 show that conspicuous changes in all parameters occurred within 1–2 days after the dietary substitution was made. Within 2 days after introduction of solid food to animals on Metrecal, DNA content was restored to that of animals on the "chow regimen," and by 5 days was even higher than that of older (42 day old) "chow-fed" rats (Fig. 1). This apparent "overshoot" was no longer evident at 10 days. At this time, total parotid DNA of animals on "chow" and "Metrecal-chow regimens" were not significantly different from each other (506 μg $\pm$ 24, 14 rats, and 520 μg $\pm$ 23, 10 rats, respectively).

Mitotic activity was markedly enhanced following substitution of the liquid by the solid diet. In the Metrecal gland, evidence of mitosis in the 32-day-old parotid was virtually absent (1 $\pm$ 0.6, per 1000 acinar cells, 10 rats) and even in glands of litter mates on the "chow regimen," mitotic rate at this age was not high [4 $\pm$ 0.5, 1000 (9 rats)]. After only 1 day of feeding solid chow to the rats previously maintained on Metrecal, mitotic activity rose, reaching levels of 10 $\pm$ 3.5 per 1000 (11 rats). The most marked increase in number of mitoses was observed at 2 days

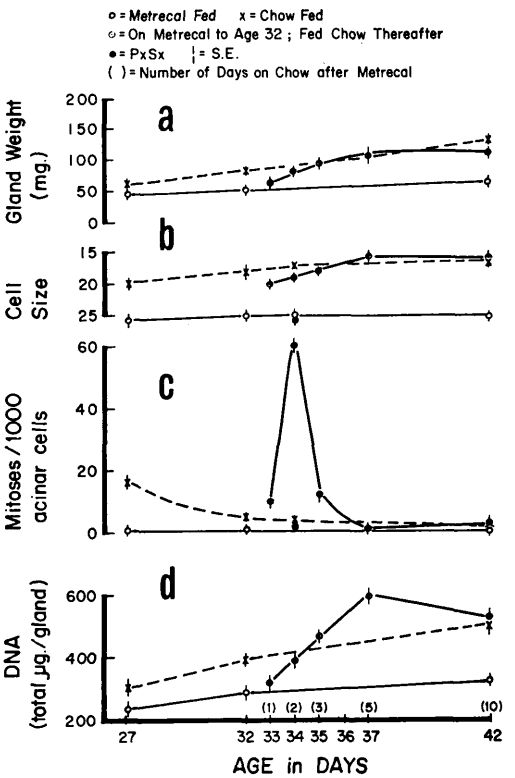

FIG. 1. Time course of change in gland wt (a), cell size (b), mitotic rate (c), and DNA content (d) of parotid of immature rats maintained on solid chow (x), or liquid Metrecal (O) from 21 to 42 days of age. In some groups of rats, at 32 days of age, a solid diet replaced the liquid diet. In such animals the postganglionic innervation to the parotids was, in some cases, intact (⊙), or in others, unilaterally severed (●) (PxSx). The time course of change in the above mentioned parameters following 1, 2, 3, 5, and 10 days of the solid diet, is shown for these 2 groups. Each point represents the mean $\pm$ SE of values obtained from no less than 9 and no more than 23 rats. Cell size was computed from microscopic examination of histological sections. Nuclear size was constant (about 6 μ in all cases) and therefore, counting number of acinar nuclei per area for each of 60 areas/rat served as a measure of cell size.

when the mean number per 1000 acinar cells was 60 ± 3 (23 rats) (a 15-fold increase over values observed in animals of the same age on "chow regimen") (Fig. 1). Thereafter, mitotic rate dropped to 12 ± 4, per 1000, (9 rats), and by 5 days was virtually identical to that of the rats that had been continuously maintained on chow from weaning (about 1–3 per 1000 cells).

Cell size, estimated by comparing number of nuclei (constant at about 6 μ) per calibrated field, exhibited a gradual increase, except for the first 24-hr period, where the increase was very marked. Here the number of nuclei per area dropped from 26 ± 0.5 (10 rats) in glands of rats on Metrecal to 20 ± 0.4 (11 rats), in those on the Metrecal-chow regimen. By 5 days after substitution of the liquid by solid food, size of parotid cells in the animals on Metrecal-chow regimen was not distinguishable from those on the chow regimen.

The data also show that neural regulation is involved in the growth changes observed following chow feeding to rats previously maintained on Metrecal. Five to 10 days after initiation of the Metrecal regimen in 20-day-old rats, complete postganlionic denervation of one parotid gland was performed in each animal. At varying intervals thereafter (immediately, 1, 4, and 7 days later), the rats were then fed solid chow for 2 days (the period found to be that of maximal mitotic activity in the "Metrecal-chow" rats). Feeding was initiated immediately after denervation in some experiments so that atrophic changes caused by denervation would be minimal (6, 7). On the other hand, in some experiments, lengthier periods postdenervation preceded chow feeding so that acute denervation effects would be minimal (8). In all cases, after 2 days of the Metrecal-chow regimen, mitotic activity in the denervated glands was low (1–3 per 1000 acinar cells) or absent (Fig. 1); in the contralateral normally-innervated gland, mitotic activity was very high (50–70 per 1000 cells) (12 rats). Examination of sham-operated controls (sham denervation of Metrecal-fed rats) showed that neither surgery *per se* nor ether stimulation produced appreciable mitotic activity.

Discussion. It is clear from the present work that the increased DNA content of parotid, observed with the introduction of a solid-food regimen to rats previously maintained on liquid ration (3), reflects an increased cell number that is due to increased cell proliferation rather than decreased loss of cells. Mitotic activity of Metrecal glands is very low but within 2 days after chow feeding begins, there is a marked increase. While mitotic activity falls rapidly thereafter, nonetheless by 5 days, DNA levels are elevated above controls and reflect the accumulation of new cells.

The present work thus shows that marked mitotic activity can be induced by increasing physiological activity of parotid gland that has been previously rendered functionally quiescent. The functional quiescence is attributed to a diminution in reflexly-mediated masticatory activity of animals on liquid, rather than solid diet, and is not the result of caloric or nutritional restriction (3, 4). Thus, with such physiological "resting" glands, the "normal" physiological activity that follows introduction of a solid diet is apparently the stimulus for the marked mitotic response elicited in the gland. Furthermore, the relative physiological quiescence of the gland appears to be an important factor predisposing the tissue to such a response. Another factor, *i.e.,* the fact that the immature gland has inherently high mitotic activity may also be involved in the marked response. The salivary glands of adult rats, on the other hand, exhibit very low levels of mitotic activity (9). Thus, although mitotic outbursts may also occur in adult salivary glands as a sequel to increased glandular activity [for example, after unilateral denervation (10), extirpation of the contralateral gland (9), or by treatment of a castrate animal with testosterone (11)], the magnitude of the mitotic response, in these cases, is usually less than that reported in the present work, and the normally low level of mitotic activity in the adult gland may partially account for the decreased response. However, the nature of the stimulus may also be a factor since even in adult salivary glands, a

marked mitotic response can be elicited under certain conditions. It is well known, for example, that the adrenergic agent, isoproterenol, can cause marked mitotic stimulation (12–14).

The present work nonetheless shows that normal glandular activity can induce mitosis and that a period of prior depressed physiological activity is apparently essential for uncovering of the link between normal physiological activity and mitosis.

It is also significant that in the absence of the autonomic nerves to the gland, no mitotic increase occurs when the solid food regimen is introduced to animals previously maintained on liquid diet. The nerves are therefore the important mediators in the functional activity that brings about the mitotic increase. Although the role of the innervation in maintenance of gland size and structure has been long recognized (6, 7, 10, 11, 15, 16), the regulatory influences of nerves on mitotic activity have been less explored (17). Thus, although the mechanism remains to be elucidated, it is significant that such neurally-induced normal physiological activity of a mammalian gland can itself evoke marked mitotic activity.

Summary. Cell size is smaller and mitotic rate and DNA content of parotid glands of rats maintained on liquid diet (Metrecal) from weaning on are lower than those of litter mates maintained on chow. At 32 days of age, when mitotic rate is 4 per 1000 acinar cells in parotid of chow-fed rats, it is nearly zero in the Metrecal rats. Substitution of a solid for liquid diet at 32 days brings about a marked increase in mitotic acitvity and DNA content, and increase in cell size, that are evident with 24 hr. The change is most marked at 1 day with regard to cell size, 2 days for mitotic rate (60 per 1000 acinar cells) and 5 days for DNA. Removal of the postganglionic innervation prevents the increase in mitosis and DNA that occurs with introduction of solid food. Neurally-mediated normal phisiological activity of the gland apparently evokes the excessive mitotic activity.

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Received Sept. 9, 1969. P.S.E.B.M., 1970, Vol. 133.