Mitotic Activity and Cell Number of Denervated Parotid of Adult Rat (37063)

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Diminution in autonomically mediated glandular activity leads to a decrease in size of rat parotid, and the degree of reduction in size is related to the kind and extent of autonomic deprivation (1). Cell size is also decreased under these circumstances but the decrease in gland and cell size are not always parallel. For example, the decrease in cell size observed when autonomically mediated glandular activity is reduced by maintenance of animals on liquid diet is somewhat greater than that observed when both postganglionic branches to the gland are surgically removed; gland size on the other hand is reduced by 50% in the latter condition and by only 35% with the liquid diet (2). Gland size, however, depends not only on size of cells but also on number of cells, and it is well known that excessive autonomic stimulation can effect increases in cell number of salivary glands (3). On the other hand, there are few reports describing cell number when autonomic influences have been decreased. Furthermore, in these few reports evidence for the autonomic effect is indirect (4, 5), or the data were obtained on immature gland where mitotic activity is normally very high (6). The present work was therefore undertaken to determine cell number in the mitotically stable adult parotid following selective elimination, mainly by surgical denervation, of autonomic influences.

Material and Methods. Female Long-Evans rats, 6 mo old, were used in these experiments and maintained on water and solid chow until initiation of experimental procedures. At this time, under ether anesthesia, unilateral excision of the superior cervical ganglion was effected (Sx), in some groups of rats, while unilateral removal of 6 mm of the auriculotemporal nerve (Px) was effected in others. In addition a combination of these two procedures was used for other groups of animals (PxSx), while still others underwent sham-operations (Sh) or were maintained exclusively on a liquid diet, either of Metrecal or milk. The denervated groups and sham-operated groups were maintained thereafter on solid chow and water *ad libitum,* for varying intervals. At these intervals, animals were anesthetized with 1% Nembutal, and the paired unstimulated parotid glands were removed, each was weighed rapidly on a torsion balance and then placed separately either in ice-cold 0.4 *N* $HClO_4$ for extraction of nucleic acids, or in Bouin's for subsequent histological examination. Sections were stained with hematoxylin and eosin and mitotic counts were made on fields containing only acini; number of cells was determined by counting number of nuclei per calibrated area as previously described (1). Mitotic counts were made on denervated and innervated glands of a pair at 1, 2, 3, 4, 6 and 8 days after surgery; with sham-operated rats, counts were made at 2, 3, and 6 days after surgery. Extraction of nucleic acids involved homogenizing the whole gland at 0–4° in 0.4 *N* $HClO_4$ and then centrifuging. The supernatant fluid which contains acid-soluble nucleotides (including ATP) was discarded. The precipitate was then dispersed and washed 3 times with cold $HClO_4$ to remove any residual acid-soluble material. The precipitate was then hydrolyzed at 90° for 15 min in 0.4 *N* $HClO_4$. Total nucleic acids were then determined by measurement of optical density at 260 nm (7, 8). Total DNA was determined using the diphenylamine reaction (9), and estimates of total RNA were obtained by subtracting DNA from total nucleic acids. Since it was shown in previous work that gland

TABLE I. Gland Weight, DNA and RNA of Rat Parotid at 4, 14 and 84 days after Denervation or Institution of Liquid Diet.[a]

Condition	No. rats	Gl. wt (mg)	DNA (μg/gland)	RNA (μg/gland)
Unop Ch, 6 mo	17	185 ± 7[b]	752 ± 35	1878 ± 81
9 mo	5	200 ± 9	767 ± 50	1985 ± 89
			4 days after denervation	
Sx	4	203 ± 7[c]	925 ± 20[c]	2146 ± 225
Con		173 ± 8	805 ± 10	1958 ± 276
Px	4	156 ± 16	759 ± 55	1276 ± 110[c]
Con		189 ± 10	745 ± 55	2070 ± 164
PxSx	4	140 ± 14	618 ± 53	969 ± 90[c]
Con		171 ± 12	618 ± 39	1925 ± 143
			14 days after denervation or liquid diet	
Sx	14	168 ± 8[c]	715 ± 15	1670 ± 58[c]
Con		182 ± 7	707 ± 21	2030 ± 78
Px	12	112 ± 6[c]	637 ± 30[c]	797 ± 68[c]
Con		174 ± 7	785 ± 31	1841 ± 69
PxSx	16	123 ± 6[c]	628 ± 29[c]	662 ± 45[c]
Con		198 ± 9	806 ± 29	2154 ± 125
Unop ME	8	126 ± 7[c]	692 ± 20	1205 ± 56[c]
Unop MI	8	113 ± 6[c]	667 ± 24	951 ± 19[c]
			84 days after denervation	
Sx	4	232 ± 11	743 ± 38	
Con		225 ± 22	711 ± 88	
PxSx	5	150 ± 4[c]	572 ± 20	
Con		250 ± 26	752 ± 65	

[a] Refers to days after unilateral denervation involving removal of superior cervical ganglion (Sx); auriculotemporal nerve (Px); combination of these two procedures (PxSx); or after institution of liquid diet regimen of liquid Metrecal (ME) or milk (MI) to unoperated rats; Con in each case refers to innervated contralateral member of pair; *Unop Ch* are innervated glands of unoperated rats (6 or 9 mo of age), maintained, like operated rats, on solid chow. Gland weight, DNA and RNA of innervated glands of sham-operated rats were, at either 4 or 14 days after surgery, not different from those of innervated glands of unoperated rats (at least 3 rats were used for each group).

[b] $M \pm$ SE.

[c] Value is significantly different ($p < .01$) from contralateral innervated gland and also innervated glands of unoperated rats.

enlargement with sympathectomy was not evident until 4 days after denervation, DNA and RNA were not determined until 4 days after denervation or sham surgery. These parameters were also determined after 14 days of denervation or sham surgery, and after 14 days of maintenance of rats on liquid diet. Only selected groups were used after 84 days of denervation and in these cases, only gland weight and DNA were determined. Innervated glands from unoperated rats at 6 and approximately 9 mo of age were also removed and examined for gland size, DNA, RNA, and mitotic index.

Results. Four days after unilateral removal of the superior cervical ganglion, values for gland weight and total DNA of the denervated parotid of adult female rats were significantly higher (about 15%) than those of the contralateral innervated glands. RNA was not, however, significantly different ($p > .01$). On the other hand, in parasympathectomized or completely denervated glands, organ weight and total DNA were not significantly different from those of the innervated mates. However, total RNA of these denervated glands was significantly lower than contralateral controls ($p < .01$) (Table I). In sham-operated rats, these parameters were not affected.

Two weeks after unilateral removal of the

parasympathetic and sympathetic postganglionic fibers to the parotid, total DNA of denervated glands was consistently less than that of the innervated mates. The mean difference between DNA of denervated and contralateral innervated glands was approximately 22% (Table I). The difference was somewhat less when comparison was made with innervated glands of unoperated rats (16%).

Removal of the parasympathetic innervation alone resulted, after 2 wk, in a reduction in DNA of 16–18% (Table I). This was generally similar in magnitude to that observed with complete postganglionectomy. With sympathectomy of 14 days duration, DNA of the denervated glands did not differ from that of innervated mates (Table I). In glands of sham-operated rats (14 days after surgery) or those of rats maintained on liquid diet (milk or Metrecal) for 14 days, DNA content was not significantly different from that of unoperated rats maintained on chow (Table I).

Total RNA of PxSx glands was markedly reduced (by about 71%) from that of the innervated contralateral gland or innervated glands of unoperated rats (Table I). On the other hand, maintenance of animals on liquid diet resulted in only a 55% decrease in total RNA when comparison was made with a group of rats maintained on solid food (Table I). This decrease was similar in magnitude to that induced by parasympathectomy alone (Table I). With sympathectomy the decrease in total RNA was only 12%.

The changes in gland weight after 14 days of denervation or use of liquid diet generally reflected the concomitant changes in DNA and RNA. Thus, with PxSx, Px, or liquid diet, gland weight showed reductions of 32–43% when comparison was made with innervated glands, but Sx glands showed only about 10% reduction.

After 84 days of denervation, gland weight and DNA of selected groups were examined to see if the earlier (14 days) atrophic effects had been superseded by regenerative changes. The two chosen represented the extremes of change: PxSx, where atrophy was maximal, and Sx where it was minimal. DNA levels, of PxSx and Sx glands as well as the innervated mates of such glands, remained at the same levels exhibited after only 14 days of denervation (Table I). Thus, DNA of PxSx glands remained reduced while that of Sx glands again did not differ from that of innervated glands. The levels of contralateral innervated glands also did not differ from 14 day levels (Table I). On the other hand, while gland weight increased somewhat in all glands, the weight of the PxSx glands remained significantly less ($p < .01$) than that of innervated glands from rats of the same age (Table I).

Since, after 4 days of denervation, DNA and gland size of the Sx glands were elevated above values recorded for the normally innervated glands (contralateral or unoperated), it was considered important to examine the mitotic activity of denervated glands in the first few days following denervation. When comparison was made with innervated glands of unoperated adult rats maintained on solid chow, increases in mitotic activity were recorded for denervated as well as contralateral innervated glands (Table II). These changes were related to the kind and duration of denervation (Table II). It is clear from the data that most marked changes occurred when denervation included removal of a superior cervical ganglion. In these instances (Sx alone as well as PxSx), both innervated and denervated glands exhibited increases in mitotic rate. The denervated gland, however, showed the most marked changes in magnitude. The mitotic rate was higher after 2 days, and reached maximal levels of 19 $\pm$ 3 per 1000 acinar cells in Sx glands, and 15 $\pm$ 3 per 1000 in PxSx glands. Sharp decreases occurred thereafter (0–3/1000), but some mitotic activity was still evident 4 days after denervation (Table II). The increases in the innervated member of the pair were comparatively small and at 2, 3 or 4 days after denervation, mitotic rate was only 2–3/1000 acinar cells. Mitotic activity was not evident in either denervated or innervated glands 6 or 8 days after surgery.

With unilateral parasympathectomy (Px), neither denervated nor innervated glands showed appreciable mitotic activity following

TABLE II. Time Course of Mitotic Changes in Denervated and Contralateral Innervated Parotid of Rats Maintained on Solid Food.[a]

After denerv. (days)	Mitoses/1000 acinar cells								
	Sx	Con		Px	Con		PxSx	Con		Unop Con
0										0.01 ± 02 (7)
1	1 ± 0.7	1 ± 1.0	(3)	0 ± 0.5	0	(3)	0 ± 0.5	0	(3)	
2	19 ± 3.4	2 ± 0.4	(12)	0 ± 0.1	0	(14)	15 ± 3.1	2 ± 0.7	(13)	
3	1 ± 0.5	3 ± 1.2	(7)	1 ± 0.4	2 ± 2.0	(6)	0	1 ± 0.4	(4)	
4	3 ± 1.0	2 ± 0.6	(6)	1 ± 0.5	1 ± 0.5	(4)	0	2 ± 1.5	(4)	
6	0	0	(3)	0	0	(2)	0	0	(2)	
8	0	0	(4)	0	0	(2)	0	0	(2)	

[a] No. of rats is given in parentheses. Sham-operated rats at 2, 3, and 6 days after surgery showed little or no mitotic activity. Days after denerv. refers to days after unilateral denervation involving removal of superior cervical ganglion (Sx); auriculotemporal nerve (Px); or combination of these two procedures (PxSx); Con in each case refers to innervated contralateral member of pair; Unop Con are innervated glands of unoperated rats, maintained, like the operated rats, on solid chow.

the surgery. A very slight increase in the denervated glands was noted 3 days after parasympathectomy; at the same time, the contralateral gland exhibited a somewhat greater burst of mitosis (Table II). Thereafter mitotic activity was not evident in either member of the pair.

Little or no increase in mitotic activity was observed in either sham-operated glands or the contralateral gland of these same animals. No mitotic activity was seen in animals on all-liquid diet at 1, 2, 3 or 4 days after institution of this regimen.

Discussion. The effects of sympathectomy on cell number of parotid of adult rat may be described as biphasic. In the early phase (1–4 days after denervation) gland size increases, and this is at least partially accounted for by the increase in mitotic activity and total DNA which occur. After this early phase, gland size decreases (10). This change is consistent with the observed decrease in DNA to normal levels and with the previously reported reduction in cell size (10). Cell number is thus not reduced by sympathectomy, even of long duration (84 days).

Parasympathectomy on the other hand not only causes a reduction in gland and cell size (3), but also a modest reduction in cell number from that of innervated glands. This reduction is, however, not apparent until 14 days after denervation. The observation that DNA of Px glands is reduced to the same extent as that of PxSx glands is consistent with the view that the parasympathetic innervation is solely responsible for the change in cell number. Furthermore, without the parasympathetic innervation, cell number remains permanently reduced (levels still low after 84 days). In the immature gland, while the same general effects are observed (6), the parasympathetic innervation exerts a more marked effect on cell number than it does in the adult. Since the parotid in the immature rat is a rapidly dividing system (11, 12) while that of the adult is a mitotically stable system (3), it may be concluded that the parasympathetic influence on the nondividing system is less pronounced than its influence on the dividing system.

Maintenance on liquid diet also leads to a decrease in parasympathetically mediated effects (13). Since the decrease in gland size effected by this regimen is not accompanied by a decrease in cell number (2), it would appear that an effect on cell number appears only when the parasympathetic pathway has been surgically eliminated. However, the explanation for these differences more probably is related to the finding that all parasympathetically mediated influences are not eliminated with liquid diet (14). Thus, it is possible that a sufficient level of parasympathetically mediated influence (either activity or trophic) remains with liquid diet to maintain cell number.

The differences between DNA levels of the denervated and innervated glands in the same

animal are more marked than differences between DNA levels of denervated glands and innervated glands of unoperated animals. The well-known compensatory enlargement (15) of the contralateral innervated gland can account for these differences. The present data also show that this compensatory enlargement is evident only when parasympathetic denervation is involved. The decreased mass of the denervated gland could impose an increased physiological load on the innervated gland, leading ultimately to its enlargement. Humoral factors released into the blood following tissue loss caused by denervation (16) may also be involved as causative factors in compensatory enlargement, and in the mitotic activity induced in denervated glands also. However, other factors must be involved in the mitotic activity induced in denervated glands since the appearance and degree of mitotic activity in such glands are dependent on the specific kind of denervation. It is possible, for example, that the heightened sensitivity of the denervated gland to circulating neurotransmitter substances plays a significant role in causing the mitotic response in the denervated gland. This supersensitivity occurs within 2 days after denervation (17), the same time at which the sympathectomized gland exhibits maximal mitotic activity. Supersensitivity may also play a role in the glandular response to parasympathectomy. In this case, however, mitosis is not prominent, but no cell loss occurs. Therefore, maintenance of cell number immediately after parasympathectomy may also be a consequence of supersensitivity. The effects attributed to supersensitivity are apparently limited to the period when the altered physiological status is first manifested. This is not surprising when examined in relation to other situations where an alteration in physiological status of the gland appears to play a role in induction of mitosis (18). Thus, glands of rats maintained on liquid diet exhibit a reduced level of physiological activity (2), and respond to the modest stimulus of chewing solid food with a burst of mitosis, similar in magnitude and time of development to that observed with sympathectomy (14). The altered physiological status exhibited by ME glands as well as Sx glands may be the prerequisite for mitosis induction; in this state, the gland is then responsive to previously ineffective stimuli.

Summary. Unilateral removal of the auriculotemporal nerve to parotid of adult rat results in decrease in gland size, total DNA and RNA. Some of these changes are evident 4 days after denervation; by 14 days, decreases in all three parameters are pronounced, and remain at this level even after 84 days. If the superior cervical ganglion is unilaterally removed, weight and DNA of the denervated gland are increased 4 days after denervation, but after 14 days, DNA is decreased to levels of the innervated gland, and small decreases in RNA and gland size are seen. With either parasympathectomy or complete postganglionectomy, DNA is reduced by approximately 16% when comparison is made with innervated glands in the same animals. In view of the gland enlargement observed 4 days after sympathectomy, mitotic activity was followed during the first 6 days following denervation. Marked mitotic activity was observed in both Sx and PxSx glands, and maximal levels of 15–19 per 1000 acinar cells were recorded after 2 days; Px glands did not exhibit mitotic bursts and mitotic activity in these, as well as in the innervated glands was relatively low (1–3 per 1000). It is suggested that the altered physiological status of the glands provides the background for induction of mitosis.

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