Physiological Activity and Regulation of Growth of Developing Parotid* (34089)

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It is well known that the physiological activity of a fully developed organ has an important role in regulating size of that organ. The parotid gland, for example, shows marked atrophy (reduction in gland size as well as size of individual cells) when reflexly mediated activity has been reduced, as with maintenance of rats on a liquid diet (1–3). The role of physiological activity in regulation of size of the immature organ is, however, less well understood. This may in part be attributed to the complexity of the growth characteristics of immature structures. In many organs, cell size and cellular proliferation continue to increase postnatally; in some organs, *e.g.*, rat parotid, differentiation as well as increases in cell size and cell number also continue postnatally (4,5). The rat parotid gland is, therefore, an ideal organ for study of regulatory influences of physiological activity on size of the organ as well as on the individual cellular components contributing to the growth. Accordingly, in these investigations the effects of diminished glandular activity (accomplished by continued maintenance of rats on a liquid diet) on gland size, cell size, and cell number were examined.

Materials and Methods. Ten-week-old male Fischer strain rats and weanling (21 days) male and female Long-Evans strain rats were used in these experiments. Rats were maintained on a ration of solid laboratory chow and water or liquid Metrecal[1] *ad libitum*. Metrecal was dispensed from a special container which required only licking for consumption (1). Before sacrifice, Fischer strain rats were fasted (water available) for 18 hr; Long-Evans strain rats were not fasted. Rats were anesthetized with pentobarbital and sacrificed by exsanguination prior to dissection of the parotid glands bilaterally. One gland was weighed and transferred to ice-cold 0.4 N $HClO_4$ for immediate nucleic acid determinations. The other gland was weighed and portions were placed in Bouin's fixative for histological section. Total nucleic acids (TNA) were extracted from the gland homogenate as previously described (5), and they were determined with a Hitachi spectrophotometer at 260 mμ (6). Total DNA was determined using the Burton (7) modification of the diphenylamine reaction and total RNA was estimated by subtracting DNA from TNA. Acinar cell counts served as a measure of cell size (1) and were made from histological sections stained with hematoxylin and eosin.

Results. Parotid gland weight, acinar cell size, and total RNA content were markedly reduced when rats were maintained on a diet of liquid Metrecal (Table I). Total DNA content, however, was not changed. The reductions in gland weight, cell size, and RNA content did not become apparent until nearly 3 days after institution of the Metrecal diet. After this time, the reductions were progressive, and by 7 days they were nearly maximal for weight (34%), cell size (52%), and RNA content (55%). When Metrecal-fed

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TABLE I. Effects of Liquid and Solid Diets on Weight, Cell Size, and Nucleic Acid Content of Adult Rat Parotid.[a]

Diet	No. rats[b]	Days of experimental diet	Body wt (g)	Parotid wt (mg)	Acinar cell count (cells /unit area)	Total DNA[c] (μg)	Total RNA[d] (μg)	RNA / DNA
Chow	7	0	195 ± 7	133 ± 7		517 ± 19	1559 ± 109	3.0
Metrecal	6	2	194 ± 4	125 ± 7		512 ± 23	1395 ± 79	2.7
Metrecal	7	3	192 ± 3	116 ± 6		609 ± 35	1270 ± 110	2.1
Metrecal	8	5	189 ± 4	92 ± 4		514 ± 15	861 ± 58	1.7
Metrecal	8	7	188 ± 5	88 ± 5	29.0 ± 0.5	539 ± 35	859 ± 68	1.6
Metrecal	8	14	211 ± 5	80 ± 6		505 ± 40	789 ± 44	1.6
Chow	7	14	223 ± 5	150 ±11	14.0 ± 0.5	544 ± 38	1757 ± 157	3.2
Metrecal-chow	9	7; 7	216 ± 3	156 ± 7	13.0 ± 0.4	557 ± 27	1832 ± 36	3.3

[a] Mean ± SE.
[b] Fischer strain rats.
[c] Colorimetric determination by diphenylamine reaction.
[d] Estimated as difference between DNA and TNA (determined by spectrophotometric analysis at 260 mμ).

rats were refed with solid chow for 1 week, parotid weight, acinar cell size, and total RNA content were restored to levels observed in animals maintained only on solid chow.

In the immature rat, the parotid of animals maintained on chow showed rapid growth (Table II). Gland weight, acinar cell size, total RNA, and total DNA content increased markedly from 21 to 42 days of age. On the other hand, when immature rats were maintained on liquid diet from 21 to 42 days of age, gland weight, cell size, and total RNA content continued to increase, but the extent of the increase, especially with cell size, was substantially less than that of chow-fed controls. Furthermore, in contrast to adult parotid, the total DNA content of parotid of immature rats maintained on Metrecal was substantially less (34%) than that of immature rats maintained on chow. The Metrecal diet did not, however, result in a complete inhibition of cellular proliferation, since total

TABLE II. Effects of Liquid and Solid Diets on Weight, Cell Size, and Nucleic Acid Content of Developing Rat Parotid.[a]

Age (days)	No. rats[b]	Diet after weaning at 21 days	Body wt (g)	Parotid wt (mg)	Acinar cell count (cells /unit area)	Total DNA[c] (μg)	Total RNA[d] (μg)	RNA / DNA
21	8		35 ± 1	39 ± 1	28.0 ± 0.3	296 ± 8	348 ± 18	1.3
32	9	Chow	76 ± 2	97 ± 4	16.2 ± 0.3	454 ± 24	1578 ± 76	3.4
42	5	Chow	118 ± 4	120 ± 7	15.9 ± 0.4	566 ± 66	1610 ± 191	2.8
Adult	7	Chow	223 ± 6	203 ± 8	13.6 ± 0.4	752 ± 31	2071 ± 125	2.8
32	14	Metrecal	74 ± 3	49 ± 2	24.8 ± 0.5	302 ± 12	721 ± 61	2.4
42	6	Metrecal	107 ± 5	69 ± 5	24.9 ± 0.3	374 ± 12	653 ± 81	1.8
Adult	5	Metrecal 14 days	216 ± 4	128 ± 4	24.6 ± 0.3	720 ± 34	1068 ± 163	1.5
42	10	Metrecal 11 days; chow 10 days	119 ± 7	158 ±10	16.0 ± 0.4	679 ± 19	2324 ± 159	3.4

[a] Mean ± SE.
[b] Long-Evans strain rats.
[c] Colorimetric determination by diphenylamine reaction.
[d] Estimated as difference between DNA and TNA (determined by spectrophotometric analysis at 260 mμ).

DNA content continued to increase with age. The effect on growth induced by liquid diet was reversed when weanling rats were fed a diet of Metrecal from 21 to 32 days of age and thereafter were fed solid chow to 42 days of age. Gland weight, cell size, total RNA content, and total DNA content of parotid generally resembled the values of control animals maintained on chow without interruption and even tended to be slightly greater.

Discussion. Previous work has shown that reduction of physiological activity of the parotid gland of mature rats by maintenance of animals on a liquid diet results in gland atrophy. This atrophy consists of a decrease in size of the whole gland and of constituent acinar cells (1). The present work shows that the gland decrease can be almost wholly attributed to the decrease in size of the acinar cells and not to a decrease in the number of cells. Total DNA content of glands from rats maintained on a liquid diet was not significantly different from that of glands of chow-fed rats even though gland weights of the two differed markedly. Furthermore, the reduction in gland weight of the Metrecal-fed animals can be accounted for by the magnitude of decrease in acinar cell size. On the basis of direct measurements of cell size, and assuming that each cell is cuboidal, the computed decrease in total acinar cell volume is 65%. If the normal gland consists of 65% acinar cells (8), this would produce a 42% decrease in whole gland weight which compares favorably with the observed decrease of 40%. Nevertheless, since adult male rats continue to grow slowly, it is possible that DNA is reduced with liquid diet. The extent of the possible decrease, however, may be so small that it cannot be measured by the techniques employed.

In the developing parotid gland, however, maintenance of animals on a liquid diet past the time of weaning appears to result in a reduced number (DNA content was lower) as well as size of cells. Even though the total number of cells in the immature parotid continued to increase with age, this increase did not occur as rapidly or to the same extent as in rats maintained on chow.

The size of the acinar cells of immature parotid in Metrecal-fed rats also continued to increase with age. The extent of the increase, however, was small. Thus, although gland growth was impeded in these Metrecal-fed rats (cell number, cell size, and gland size were reduced), cessation of growth did not occur. This conclusion is in opposition to that advanced recently by Wells and Peronace (3). They suggested that, while liquid diet reduces parotid weight in adult rats by causing a true atrophy, in growing rats it resulted instead in a cessation of growth. Their data, however, were insufficient to support the contention that maintenance of growing rats on liquid diet causes cessation of growth. The present study shows that, in growing rats maintained on liquid diet, not only does a small increase in acinar size occur, but cell number also continues to increase. Maintenance of the immature rat on a liquid diet beyond the time of weaning thus appears to result in an inhibition, but not a cessation, of parotid growth.

Liquid diet in the young rat, as in the adult, is considered to result in a diminished physiological activity of the gland, and it is this decreased glandular activity that apparently causes the effects on growth. Thus, physiological activity apparently has an important role in regulation of the growth of a developing organ. However, whether physiological activity is a direct or an indirect mediator of this regulation remains to be elucidated. Since the autonomic innervation regulates glandular activity, it is possible that it is the autonomic mediators themselves which directly affect growth. It has been shown, for example, that the adrenergic agent, isoproterenol, markedly accelerates growth and differentiation of the postnatal parotid gland (9), and Drachman (10) has presented evidence for a trophic effect of acetylcholine on developing muscle.

Since, in the growing gland, reduced physiological activity resulted not only in a decreased cell size, but also in a reduced number of cells, it was important to determine whether return to a normal level of glandular activity would restore gland size to the levels that would normally have existed had there been no intervening period of di-

minished activity. It is clear that not only gland and cell size are restored to expected levels by reinstitution of a solid diet, but cell numbers are also restored. In fact, there is an "overshoot" in cell number (DNA content of the Metrecal-refed rats is even higher than that of rats maintained continuously on chow). Thus, although the data suggest that increased physiological activity of the gland may lead to increased cellular proliferation, direct evidence on this point is presently lacking.

Summary. Physiological activity of the parotid gland was reduced by maintenance of immature and adult rats on a liquid diet. The general status of the gland was determined by measurements of weight while the status of the individual cellular components was determined by measurements of cell size and nucleic acids. In the developing parotid, reduced physiological activity inhibited the growth of the gland by inhibiting growth in both cell number and cell size. In the mature parotid, reduced physiological activity resulted in a decrease in cell size only. When glandular activity was returned to normal by feeding solid food in both the immature and adult rat, the gland and its cellular components were restored to expected levels.

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