Increased Chromosome Number of Rat Parotid Cells After Isoproterenol.* (32189)

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The increased size of salivary glands caused by administration of pharmacologic doses of isoproterenol to the whole animal is generally attributed to hyperplasia and hypertrophy(1-7). However, the relative role of each of these processes as well as the condition of the chromosomes of cells in the enlarged glands has not been delineated. In the present investigation, therefore, measurements of gland enlargement, mitotic rate and chromosome count have been made in an attempt to elucidate the processes involved in gland enlargement generally, and, specifically, in elargement caused by prolonged action of an autonomic agent.

Materials and methods. Female Long-Evans rats, 5-7 months of age, were used in these investigations. Twice daily administration of 6 mg doses of isoproterenol HCl in volumes of 0.3 cc distilled water was made by i.p. injection. Control animals were either untreated or injected with equal volumes of aqueous NaCl solution of similar osmolarity and pH as that of the drug solution. Animals were maintained on this regimen for periods varying from 1 to 28 days, while being provided access to drinking water *ad lib.* Eighteen hours prior to sacrifice and removal of tissues, injections were stopped and food was removed from the cages. Just before sacrifice, a parotid gland was removed, under Nembutal anesthesia, and quickly weighed on a torsion balance. Sections were preserved either in Bouin's for subsequent histological examination (with hematoxylin and eosin staining) or in formalin for subsequent Feulgen staining(8). Other pieces were separately weighed, and placed in Hanks' solution, and subsequently minced finely with scissors for squash preparations of chromosomes. Aceto-orcein(1%) staining was employed, after treatment of the cell suspension with 0.7% sodium citrate and a fixative consisting of 5 parts acetic acid, 4 parts water, 1 part 1N HCl. Phase microscopic examination of chromosome preparations was employed for estimating chromosome number. Mitotic rate was determined by counts of mitoses per 1,000 cells; determination of total number of diploid (2n) and greater-than-diploid (>2n) cells per slide (of approximately 1000-5000 cells) was separately made. Small tissue biopsies (2 mm) were used for cell culture initiation by mincing this tissue in a growth medium (20% calf serum, 1% chick embryo extract, 29% Hanks' BSS, 50% TEM) and incubating in a 5-8% CO_2 atmosphere. After several days of growth colchicine (final concentration 10^{-6} M) was used to arrest mitosis in fibroblast subcultures 2 hours before harvest.

Results. The data in Table I show that enlargement of the parotid glands of adult female rats occurred following intraperitoneal administration of the sympathetic agent, isoproterenol (IPR), and this increase, while already quite evident after only 2 days of twice-daily injections of the agent, continued to increase with time. Although small further increases could be obtained as long as the agent was administered, gland size was nearly maximal by 8-10 days. Reversal of the enlargement could be achieved by cessation of isoproterenol injection. Ten to 14 days after IPR withdrawal, mean parotid weight (6 rats) was 336 ± 29 as compared with an untreated control group (14 rats) where the mean was 224 ± 12. After 50 days, reversal was virtually complete (258 ± 22, 5 rats).

Microscopic examination of sections of tissue prepared from the glands of rats subjected to varying periods of IPR-treatment revealed that a marked increase in mitotic activity of the acinar cells occurred in the first days after instituting this regimen (1-5 days) (Fig. 1); hypertrophy (increased cell size) of

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TABLE I. Duration of Isoproterenol Treatment and Effect on Parotid Gland Size, Mitotic Rate, and Chromosome Complement.

Days on IPR*	No. rats	Gland wt (mg)	Mitotic index†	Mitoses‡		% of mitoses§	
				2n	>2n	2n	>2n
0‖	10	206 ± 7¶	0				
1	7	244 ± 9	.8 ± .5	1 ± .5	0	100	0
2	13	359 ± 21	4.0 ± .5	2 ± .4	1 ± .3	70	30
3	13	473 ± 39	7.5 ± .8	16 ±3	14 ± 3	53	47
4	5	642 ± 56	2.0 ± .6	1 ± .8	3 ± 2	25	75
5	4	929 ± 52	.5 ± .2	0	1	0	100
6	4	858 ±162	>.5	0	1	0	0
7	2	890	0	0	0	0	0
8	6	1208 ±201	0	0	0	0	0
10	9	1186 ± 97	0	0	0	0	0
12	2	1379	0	0	0	0	0
14	2	1531	0	0	0	0	0
18	3	1400	0	0	0	0	0
28	3	1961 ±263					

* 12 mg IPR administered i.p. in twice-daily doses of 6 mg in .3 cc H_2O.

† Mitoses per 1000 cells counted. Cells were counted from 3-5 separate slides for each rat.

‡ Total number of diploid (2n) and greater-than-diploid (>2n) cells per slide (1000-5000 cells usually found per slide); counts made from 3-5 separate slides for each rat.

§ % of total number of mitoses that are 2n and >2n.

‖ Uninjected controls. In addition, 9 control rats (mean gland wt = 203 ± 8), injected i.p. twice daily with .3 cc aqueous NaCl (isosmotic to IPR) for 2, 3 or 4 days, showed virtually no mitotic activity (<.01 per 1000 cells counted).

¶ M ± S.E.

the acinar cells was also initiated during this early period and continued for the duration of the IPR treatment, although cell size increased to nearly maximal limits within 6-8 days, and thereafter the increased size was maintained (Fig. 1). Mitoses, however, were not generally observed in the later periods (after 6 days), although in some sections, mitoses (1 or 2 per 1,000 cells) were found as late as 8 (and, very infrequently, even at 10) days after initiation of IPR treatment. The mitotic rate at 3 days (in sections from this series) was approximately 16 per 1,000 cells (6 rats).

Squash preparations of cells from the minced tissue from parotid gland confirmed the fact that the most extensive proliferation of the acinar elements was confined to the first few days following initiation of isoproterenol treatment. Mitotic rate, determined from phase microscope examination of the preparations, revealed a marked increase over normal control values in number of mitoses after 2 days of treatment. Virtually no mitotic figures were recorded in controls injected with aqueous NaCl solution (2-4 days) or those not injected at all. In the IPR-treated glands, after 1 day of treatment, only 1 mitotic figure was found per 1,000 cells counted; after 3 days, the rate had increased to 4/1,000 and by 3 days, a maximal rate of 7.5/1,000 cells was recorded. By 6-8 days mitotic activity was not usually seen (Table I). Furthermore, counts of the chromosomes of these cells revealed that, after 1 day of IPR, all of the cells were diploid (42 chromosomes); after 2 days, while most of the cells were diploid, at least 1/3 to 1/4 were greater-than-diploid (usually 84 chromosomes) and after 3 days, as many as 51 diploid and 48 greater-than-diploid figures were found in 4,000-5,000 cells counted (total number of cells per slide) (Fig. 2). At 4 days the ratio of 2n to >2n was altered, and most of the cells here were >2n (1:4). By 6-8 days, mitotic figures were not observed in the squash preparations.

Attempts to assess the chromosome complement of the hypertrophied cells were made by observation of cultures of cells obtained from glands 14 days after initiation of isoproterenol treatment. Microscopic examination at this time showed hypertrophy and little or no mitotic activity either in H and E sections, or in the squash preparations. In

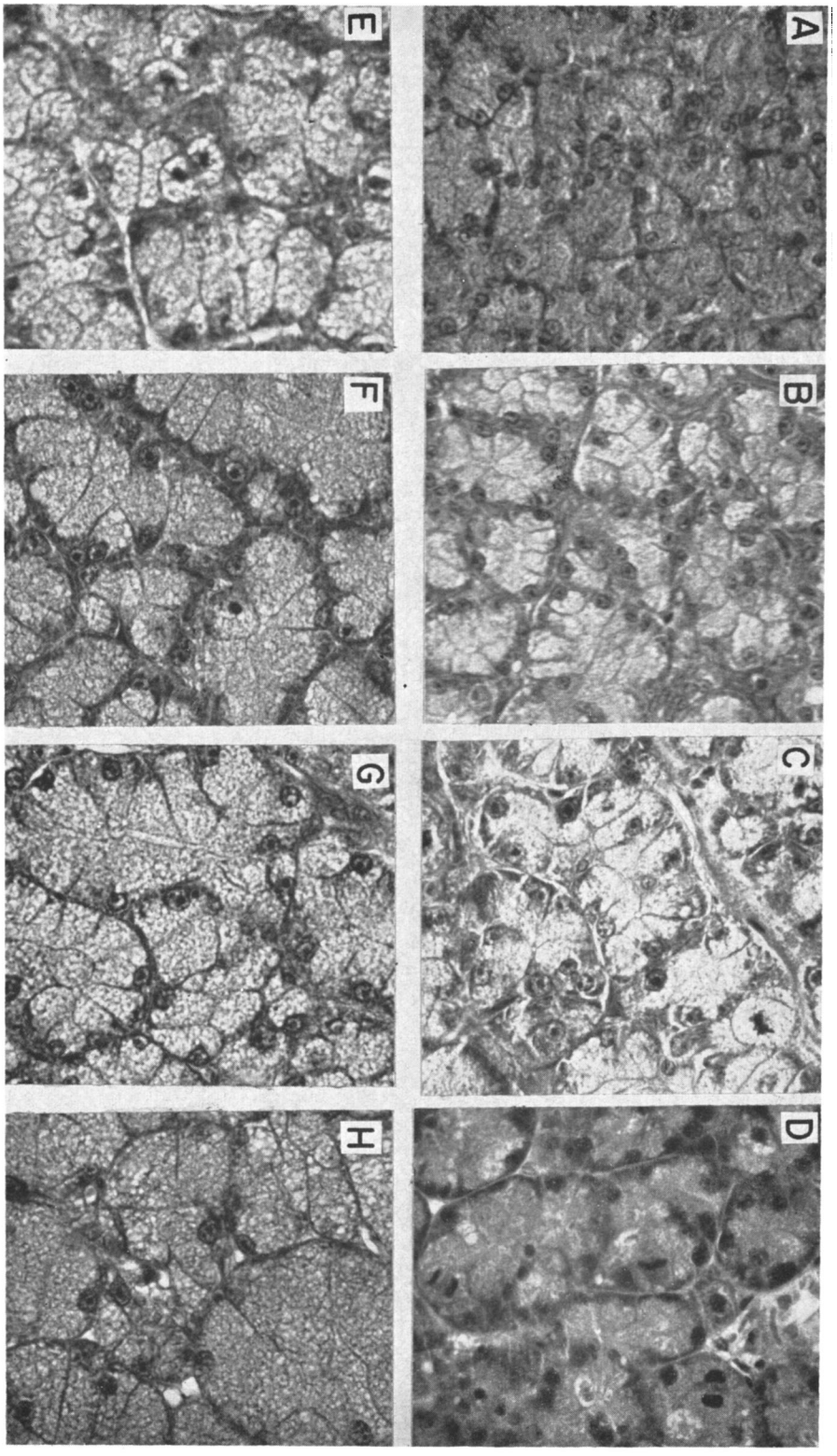
A
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FIG. 1. Haemotoxylin and eosin stained sections of parotid gland. (A): Normal rat parotid. (B) thru (H): parotid gland after 1, 2, 3, 4, 5, 6 and 10 days of isoproterenol administration. Note progressive increase in size of acinar cells and nuclei, with time. Mitotic activity is most pronounced after 3 days (D) with at least 6 conspicuous mitotic figures in area shown; in (C), (E), and (F) mitoses are also seen but not in (A), (B), (G), or (H). Mag. × 333.

several successive transfers, growth of the cells was achieved, but the chromosome complement of the growing cells was diploid. A karyotype of a fibroblast derived from culture of a gland previously treated with IPR for 3 days is shown in Fig. 3. Cultures and subsequent transfers of the cells from glands maintained on IPR for 2, 4 or 8 days showed

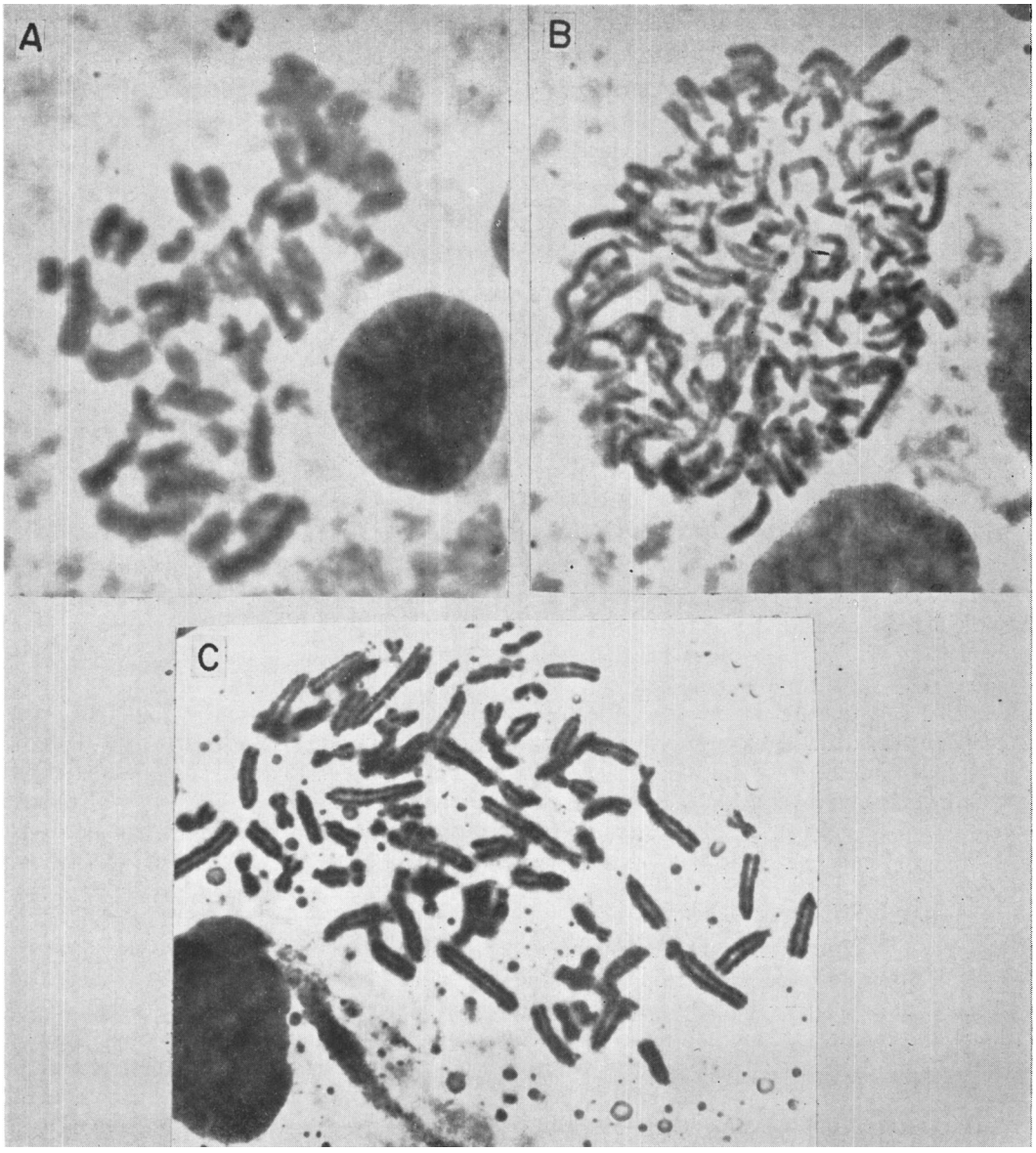

FIG. 2. Metaphase chromosome plates prepared from squash preparations of parotid tissue from 3 day IPR-treated rats. (A): Diploid chromosome configuration; (B) and (C): Greater-than-diploid chromosome number. Mag. × 1466.

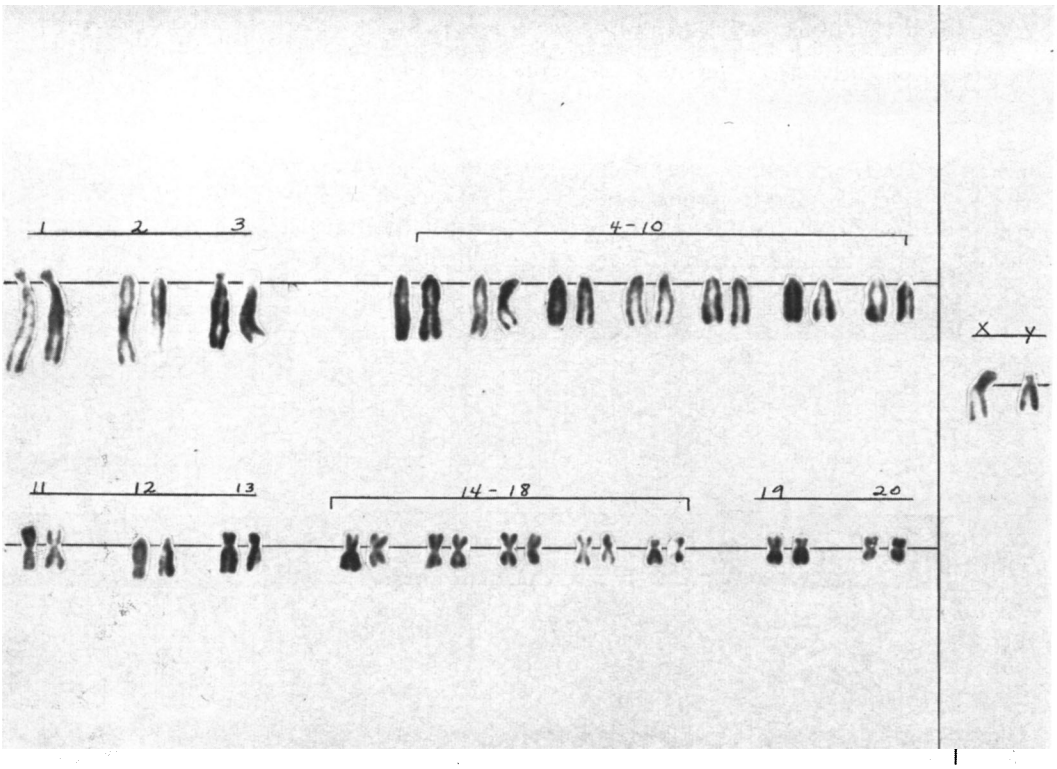

FIG. 3. Karyotype of a fibroblast derived from culture of a 3-day IPR-treated parotid gland, showing 42 chromosomes. Mag. × 1000.

diploid cells. Mitotic activity was absent in tissue from "reversed" glands.

Measurement of nuclear size was also employed to assess the chromosome complement of the hypertrophied cells. Nuclear diameter of cells from normal parotid gland averaged 6.5 μ; after 10 days of IPR treatment, nuclear diameter was approximately 11 μ and at the intermediate time of 3 days nuclear diameter averaged 8 μ. This 3-day value was a reflection of the normal-sized (6-7 μ) and enlarged nuclei (11 μ) that were observed.

In addition, the Feulgen nucleal reaction was used for estimation of DNA content(8). Such staining of the hypertrophied tissue revealed a number of densely-stained nuclei that were larger and more intensely-stained than those of nuclei from normal control glands. The estimates of DNA content were made from visual examination of photomicrographs of stained tissues and were therefore approximate; quantitative spectro-photometric data were not obtained.

Discussion. It was originally suggested by Selye *et al* that the salivary gland enlargement caused by prolonged administration of isoproterenol to rats was attributable to hyperplasia as well as hypertrophy of the acinar cells(1). Later work attributed the increase principally to hypertrophy(2,3,4). More recently Seifert(5), Barka(6), and Pohto(7) have suggested that hyperplasia exerts the prominent role in effecting the enlargement. Furthermore, Barka has shown that IPR stimulates DNA synthesis of the rat submaxillary gland and that a parallel increase in mitotic activity occurs(6); Pohto has also found an increase in DNA content of IPR-treated rat parotid glands(7). The present work suggests that both processes are important in bringing about the IPR-induced gland enlargement but also that each process has a definite time interval during which its role is more conspicuous. The work also suggests that hypertrophy is more important in maintenance of the increased gland size during prolonged IPR-treatment. It must be pointed

out, however, that the conditions of IPR treatment may be important factors in regulation of the enlargement. Under the present conditions of experiment, dosage was significantly lower than that employed in other investigations(5,6,7) (12 mg daily as compared with 40-100 mg) and IPR administration was terminated 18 hours before sacrifice.

The mitotic rate of normal rat parotid is very low(9). The sharp increases in mitotic rate observed 2-3 days after initiation of IPR therefore suggest that cell proliferation plays a role in effecting increases in gland size during the early interval after IPR initiation. However, the mitotic activity drops soon after this, and hyperplasia is probably not significantly involved in gland enlargement after more prolonged IPR treatment. Hypertrophy, on the other hand, contributes to the gland enlargement during the entire time of IPR administration, and in fact, has a prominent role in the enlargement even in the initial days after IPR administration. This is suggested by the fact that the IPR-induced increases in weight of the gland are, to a marked extent, paralleled by increases in acinar-cell size. After 2 days of IPR treatment, the size of individual acinar cells as well as the whole gland is nearly double normal levels. At 3-4 days, the increase in gland size, while dependent on the marked increase in cell proliferation, is also related to the concurrently occurring increase in cell size. By 6-10 days, the increase in cell size (and gland size) are nearly maximal (about 6-7 times normal), and this increased cell and gland size are virtually maintained thereafter if IPR is continued.

The rapid and nearly complete return to normal gland size after withdrawal of IPR also argues in favor of the view that hypertrophy has a conspicuous role in causing gland enlargement(3). Even the increases in gland size and acinar cell size which occur early, when hyperplasia is prominent, are almost entirely reversible (from a size 6-7 times normal to 1 1/2 times normal). Thus, while hypertrophy contributes to the gland enlargement during the entire period of IPR administration, it appears that the marked increase in production of new cells is generally confined to the early phase.

To examine the relationship between the new cells produced and the hyperthrophic cells, examination of the chromosome complement of the enlarging cells by using squash preparations proved to be particularly useful, especially in the early period when mitosis was most prominent. During this initial period of 1-5 days when extensive cell proliferation and hypertrophy were occurring concurrently, both diploid and greater-than-diploid metaphase plates were observed. Furthermore, the proportion of diploid to greater-than-diploid cells varied with time in a very suggestive way, *i.e.*, initially, when mitotic rate was low, (at 1-2 days), all (or nearly all) of the cells in mitosis were diploid; by 3 days, when mitotic rate was maximal, the number of $>2n$ cells had increased markedly and, in fact, approximately equal numbers of both were observed; by 4 days, nearly all of the cells in mitosis were $>2n$, and no 2n cells at all were seen at 5 or 6 days. There was a gradual shift, therefore, in proportion of $>2n$ cells, and when hypertrophy became the dominant cytological event, only polyploid cells were observed. It can only be suggested at present, however, that this shift presages an increased complement of chromosomes in the nuclei of the enlarged cells. Attempts to assess the chromosome complement during the later period when mitosis was not usually seen met with only moderate success, and therefore only an approximate estimation of the chromosome complement of the already-enlarged cells was possible. An increase in chromosome complement was inferred by comparing nuclear size of the hypertrophied cells with that of normal cells, or cells from glands maintained for only 1-3 days on the IPR regimen. The increase in nuclear size within a given tissue is probably related to an increase in chromosome complement(10,11). An increase was also suggested by the apparent increase in density of Feulgen-positive material (and thus DNA content) in the nuclei of the enlarged cells. No quantitative determinations of DNA based on absorption were made(11), however. Finally, fragments of tissue from glands where no metaphase plates were detected in squash

preparations were transplanted to tissue culture. In these growing transplanted cells, however, only diploid metaphase plates were observed. These methods for determining the chromosome complement of already-enlarged and virtually non-dividing cells were thus only moderately successful. The evidence of polyploid chromosomes, however, and the shift in proportion of 2n and >2n cells does suggest that the enlarged cells may have more chromosomes than the normal cells. The mechanism of the increase in chromosome number may involve non-disjunction of chromosomes but proof for this remains to be obtained.

Summary. Administration of pharmacologic doses of the sympathetic amine, isoproterenol, to adult female rats resulted in a progressive enlargement of the gland with duration of treatment. Although this enlargement is the result of hypertrophy of acinar cells as well as proliferation of these elements, marked mitotic activity was generally confined to the early days of treatment (1-5 days) whereas hypertrophy was initiated almost immediately and persisted throughout the period of isoproterenol-administration. During the early phase of marked cellular proliferation, chromosome counts from squash preparations of tissue indicated that greater-than-diploid (>2n) as well as diploid (2n) cells were present. Furthermore, the proportion of 2n to >2n changed with time in a highly suggestive way, *i.e.*, at 1-2 days, when mitotic activity was increased above normal, but low, 2n cells predominated; at 3 days, when mitotic rate was maximal, equal numbers of 2n and >2n cells were observed; and at 4-5 days, when mitotic rate dropped, the >2n cells were most evident. Culturing the hypertrophic cells (14 days of IPR treatment) produced only 2n cells. Measurements of nuclei of the enlarged cells, estimations of DNA content by visual examination, and the occurrence of polyploid cells in definite ratios with time all suggest the existence of polyploid nuclei in the enlarged glands.

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Effects of Hemorrhage on Intestinal Absorption and Secretion. (32190)

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Several lines of evidence indicate there are functional similarities between the intestine and the renal tubule. Histologically both consist of columnar epithelial cells with microvilli. Both can reabsorb substances against electrochemical gradients and can create osmotic pressure differences between the interstitial space and respective lumens. There is also evidence that there is a similarity of the regulation of salt and water transport across these two organs. In dogs with thoracic vena cava

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