Effect of Dietary Deficiency in Vitamin D, Calcium, and Phosphorus on the Ultrastructure of the Rat Parathyroid Gland

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PARATHYROID HYPERTROPHY has often been observed in experimental animals with Vitamin D deficiency.[2-5] Recent studies by Au and Raisz [6] indicated that not only parathyroid size, but also functional activity as measured by the uptake of α-aminoisobutyric acid (AIB) was increased in the parathyroid glands of Vitamin D-deficient rats when the serum calcium concentration was low. In addition they demonstrated that the glands could secrete parathyroid hormone in organ culture and that the uptake of AIB into these Vitamin D-deficient glands could be inhibited in vitro by calcium in a manner similar to that of normal glands. These studies help to assess changes in the overall function of the gland but do not indicate the state of function of individual chief cells. Munger and Roth [7] and Roth and Raisz [8] described the ultrastructural variation in the chief cells of the parathyroid and correlated it with the phase of protein synthetic activity of the cells. Cells could be classified as "resting" or "active," and overall function of the gland classified as "stimulated," "normal," or "suppressed," by

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evaluating the relative number of chief cells in the various phases of the cycle.[8] The present study of the ultrastructure of parathyroid glands in rats deficient in Vitamin D, calcium, and phosphorus was undertaken to further correlate the biochemical parameters of activity with the morphologic ones.

Materials and Methods

Two sets of experimental animals were used as described below (see Table 1 for identification of groups).

Group I. Groups of 3 weanling, male, Holtzman rats were fed for 5 weeks the semisynthetic Vitamin D-deficient diets designed by Steenbock and Herting[9] as modified by DeLuca, *et al.*[10] The rats were housed in individual, hanging cages in a room maintained at a constant temperature and humidity, and free of measurable ultraviolet light. The calcium and phosphorus content of the diet was varied as follows: (IA) low Ca/P (0.03% calcium and 0.1% phosphorus); (IB) normal Ca/P (0.4% calcium and 0.3% phosphorus); and (IC) high Ca/P (0.8% calcium and 0.1% phosphorus). Control rats for each group were maintained on identical diets but given orally 80 I.U. of Vitamin D_2 dissolved in cottonseed oil every 3 days. The levels of calcium and phosphorus in the serum of each animal were determined at sacrifice (Table 1).

Group II. Male weanling Sprague-Dawley rats were divided (Table 1) into 5 groups (6 animals each). One group (IIA) was maintained on Purina Lab Chow (1.42% calcium and 0.96% phosphorus). The other 4 groups were maintained for 3 weeks on a high-calcium, low-phosphorus, Vitamin D-free rachitogenic diet (1.2% calcium and 0.1% phosphorus), as described by Harrison and Harrison.[11] Seven days prior to sacrifice the diets of three of these groups were modified as follows: (IIC) 10 I.U. of Vitamin D_2 was added per gram of diet; (IID) NaH_2PO_4 was added to produce a Ca/P ratio of 1.4:1; and (IIE) NaH_2PO_4 and Vitamin D_2 were added as in Groups IIC and IID. The fourth group (IIB) was continued on the rachitogenic diet. Seventy similar Sprague-Dawley rats were divided into 5 identical treatment groups of 10–15 animals each, and had serum calcium and phosphorus determinations performed at the time of sacrifice of the experimental animals.

Tissue Preparation. The parathyroid glands were removed rapidly, fixed in cold (0° C.) Caulfield's[12] modification of Palade's[13] Veronal-buffered 1% osmium tetroxide or Dalton's[14] 2% chrome-osmium mixture. The glands were rapidly dehydrated with graded ethanol in the cold (4° C.) and embedded in Epon 812 by the method of Luft.[15] Silver sections were cut on a Porter-Blum MT-2 ultramicrotome, with a Dupont diamond knife, mounted on bare 200-mesh copper grids, and examined, after lead citrate[16,17] or uranyl acetate[18] staining, in an RCA 3G electron microscope with 100 kv accelerating voltage.

The serum calcium levels were determined by the method of Beale and Bostrom[19] for Group I and Skeggs modification of Williams and Moser's method[20] for the Technicon Auto-Analyzer for Group II. The levels of serum inorganic phosphorus for Group I were determined by the method of Chen, *et al.*[21] and for Group II by the method of Fiske and Subbarow.[22]

Results

On the basis of an overall assessment of ultrastructural appearance, the parathyroid glands could be divided into three groups. Animals

which had persistently low serum calcium concentration showed "active" or "stimulated" parathyroid glands whether they had been on a low calcium diet with or without Vitamin D (Groups IA and IA + D) or on a normal calcium diet without Vitamin D (Group IB). The parathyroid glands of animals with normal serum calcium concentrations all appeared essentially "normal," regardless of whether the animals were Vitamin-D deficient, and whether their phosphorus intake was low (Groups IB + D, IIA, IIB, IIC, and IIE). Two groups of animals had high serum calcium concentrations and their parathyroid glands appeared "suppressed." These animals had received diets with a high calcium:phosphorus ratio plus vitamin D (IC + D and IID). The findings in Group IC (high calcium diet without Vitamin D) were mixed; 1 animal with a serum calcium of 11.4 mg./100 ml. showed a "suppressed" gland (Fig. 8). The other 2 animals, with low serum calcium levels, showed "active" glands (Fig. 3 and Table 1).

The basis of assignment to one of the three morphologic groups was as follows:

Active or Stimulated

These glands (Groups IA, IA + D, IB, and IC) were enlarged two to three times compared with controls, as previously reported by Au and Raisz.[6] The majority of the chief cells were enlarged, having tortuous cell membranes with numerous interdigitations (Fig. 1). The ribosomes were usually in small aggregates and clusters. The granular endoplasmic reticulum was largely dispersed (Fig. 2) though a few cells showed prominent aggregated arrays of parallel lamellas. The Golgi regions were usually large and tortuous, with numerous vacuoles and vesicles (Fig. 3). Mitochondria only rarely contained electron-dense small granules. The nuclei often were irregular in outline.

Normal

Normal parathyroid glands (Groups IB + D, IIA, IIB, IIC, and IIE) closely resembled in their ultrastructure (Fig. 4) the normal rat parathyroid glands (Fig. 5) examined previously[23] or cultured for 48 hr. in a normal calcium medium.[23] The cell membranes were straighter and had fewer interdigitations than those of animals of the "active" group. The chief cells were somewhat smaller than those of the "active" group. A mixture of cells was found with regard to cellular activity. The morphology revealed resting chief cells[8] with dispersed granular endoplasmic reticulum and small Golgi regions with few vacuoles and vesicles (Fig. 4), cells with the granular endoplasmic reticulum ag-

Table 1. Serum Calcium, Serum Phosphorus, Calcium:Phosphorus Ratio, and Gland Morphology of Experimental Animals

	Diet	Serum Ca (mg./100 ml.)	Serum P (mg./100 ml.)	Serum Ca/P ratio	Morphology
		Group I			
A	Low Ca/P	5.2	9.4	0.55	
		4.6	10.8	0.43	Stimulated
		4.4	6.1	0.72	
A + D	Low Ca/P + Vitamin D	7.7	9.3	0.83	
		8.2	9.4	0.87	Stimulated
		8.7	9.6	0.91	
B	Normal Ca/P	6.2	10.4	0.60	
		5.8	12.1	0.48	Stimulated
		6.7	10.4	0.64	
B + D	Normal Ca/P + Vitamin D (control)	9.4	7.9	1.2	
		10.2	9.2	1.1	Normal
		9.1	9.4	0.97	
C	High Ca/P	8.8	4.2	2.1	
		8.3	5.1	1.6	Stimulated*
		11.4	5.1	2.2	
C + D	High Ca/P + Vitamin D	11.0	6.0	1.8	
		9.9	9.8	0.99	Suppressed
		11.1	6.5	1.7	
		Group II†			
A	Normal	10.67 ± 0.22‡	10.37 ± 0.86	0.97	Normal
B	Rickets (R)	10.23 ± 0.39	2.57 ± 0.61§	4.0	Normal
C	R + P	10.34 ± 0.72	10.07 ± 1.50	1.0	Normal
D	R + Vitamin D	12.63 ± 0.22§	7.50 ± 0.86‖	1.6	Suppressed
E	R + P + Vitamin D	10.15 ± 0.45	9.76 ± 0.28	1.0	Normal

* The animal whose serum Ca concentration was 11.4 mg./100 ml. belonged in the suppressed group morphologically.
† Animals comparable to Group II.
‡ Standard deviation.
§ Significantly different from all other groups at the 0.001 level.
‖ Significantly different from all the rachitic animals at the 0.001 level.

gregated into a juxtanuclear body (Fig. 6), and "active" cells[8] with large Golgi apparatuses (Fig. 4), with numerous vacuoles and vesicles.

Suppressed

The suppressed parathyroid glands (Groups IC + D and IID) resembled glands examined after culture in a high calcium medium.[8,23] These chief cells were almost uniformly small with relatively straight cell membranes (Fig. 7). The only remaining fluting was at the corners of the cells. The ribonucleoprotein particles were somewhat more

dispersed than in the other groups (Fig. 8). Only rarely did a chief cell have its granular endoplasmic reticulum aggregated. The Golgi apparatuses of the vast majority of the chief cells were small with few vacuoles and vesicles (Fig. 7 and 8). Mitochondrial granules were rare. Occasional chief cells in the active stages of secretion were noted.

Secretory granules were rare in all three groups of glands, as has been reported in the rat parathyroid gland.[23]

Discussion

Munger and Roth [7] demonstrated that the parathyroid chief cells of the Virginia deer and of the human had a secretory cycle similar to that of the exocrine pancreas.[24] It appears that the parathyroid chief cell aggregates its granular endoplasmic reticulum transiently [8] while producing a quantity of parathyroid hormone which is then wholly secreted prior to producing more hormone. Roth and Raisz [8,23] demonstrated a similar cycle in the chief cells of rat parathyroid glands, and the secretory cycle has been demonstrated in the bovine gland,[25] in the dog, monkey, and mouse [26,27] and in amphibians.[28–30] Suppression of parathyroid activity in man,[31] the rat,[8,23,32] the cow,[33,34] and the mouse [27,35,36] induces a similar morphologic pattern, causing the majority of the chief cells to remain in the resting stage. The ultrastructure of chief cells of the rat parathyroid gland can be altered by varying the ambient calcium concentration of glands being cultured in vitro.[8,23] Decreased serum calcium concentration is correlated with an increased proportion of active chief cells in the rat[23] and cow[37] parathyroid glands.

Capen, Koestner, and Cole [33] and Capen, Cole, and Hibbs [34] demonstrated that chief cells of cows fed high doses of Vitamin D had a decrease in nuclear surface area, in cytoplasm:nucleus ratio, and in parenchyma:interstitium ratio. The cows fed high doses of Vitamin D also had decreased numbers of actively synthesizing and secreting chief cells, while immediately pre- and postpartum cows had an increased number of actively synthesizing and secreting cells. Even though the Vitamin D in the toxic doses given in this study [34] elevated both the serum calcium and the serum phosphorus, the authors felt that it must be the serum calcium which was suppressing the parathyroid glands. These studies,[33] however, could not eliminate the possibility that the Vitamin D alone would suppress the parathyroid glands, or that an increased ratio of serum calcium to serum inorganic phosphorus would suppress the glands.

Parathyroid hypertrophy [25] and increased parathyroid function [6] have

been demonstrated in certain Vitamin D-deficient states. The increased parathyroid function appears to depend upon the level of the (ionized) serum calcium.[6] In the present study, in which the serum calcium and phosphorus and the dietary Vitamin D of weanling rats were varied independently, the morphologic activity of the parathyroid glands was classified in three groups: "active" (the majority of the chief cells were in an active stage of parathyroid hormone synthesis and secretion); "normal" (the number of active and inactive cells appeared about equal to the ratio seen in the normal rat gland;[23] and "suppressed" (the number of resting cells was markedly predominant). Vitamin D did not suppress the glands directly since the glands of animals (IA and IA + D) with a low calcium intake were "stimulated" regardless of whether Vitamin D was present. Neither did Vitamin D deficiency, without a depression of the serum calcium (IIB, IIC, and one gland of Group IC), stimulate the parathyroid glands. Though depressed ratios of calcium to phosphorus in the diet cannot be eliminated as a cause of stimulation, elevated ratios, such as are seen in the high calcium:phosphorus diets without Vitamin D (one animal of Group IC and the rachitic Group IIB), did not suppress activity. Decreased serum phosphorus did not stimulate parathyroid activity, as demonstrated in the rachitic group (IIB), the rachitic group with added Vitamin D in which the glands were suppressed (IID), or the high calcium:phosphorus, Vitamin D-deficient animals (one animal of IC). This would then further support the hypothesis previously put forth [6,8,23] that the level of the serum calcium (or more precisely the ionized calcium) controls the synthesis and secretion of parathyroid hormone by the chief cells of the parathyroid gland, independently of the serum phosphorus, the ratio of calcium to phosphorus, or the Vitamin D level. Though this study gives little information as to the site of action of the calcium ion, the alterations in morphology of the stimulated and suppressed parathyroid glands are very similar to those previously seen in stimulated and suppressed glands in vitro.[8,23]

Summary

Suppression or stimulation of the parathyroid glands of the rat, as evidenced by the relative numbers of active versus inactive cells seen with the electron microscope, is controlled by the serum calcium concentration. The serum inorganic phosphorus, the ratio of dietary or serum calcium to phosphorus, the presence or absence of Vitamin D in the diet, or the presence or absence of rickets do not affect parathyroid gland function unless the serum calcium is altered.

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[*Illustrations follow*]

Legends for Figures

Figures 1–3 and 8 are of sections stained with uranyl acetate; Fig. 4–7, sections stained with lead citrate.

Fig. 1. Portions of 2 cells from an "active" gland, Group IB. The cells are large, with marked tortuosity and numerous interdigitations of the plasma membranes *(P)*. Ribonucleoprotein particles *(R)* are largely aggregated. The Golgi *(G)* apparatuses are large, with numerous vacuoles and vesicles. No secretory granules are present in this section. Mitochondria *(M)* usually do not contain mitochondrial granules. Nuclear *(N)* outlines are irregular. × 14,500.


M
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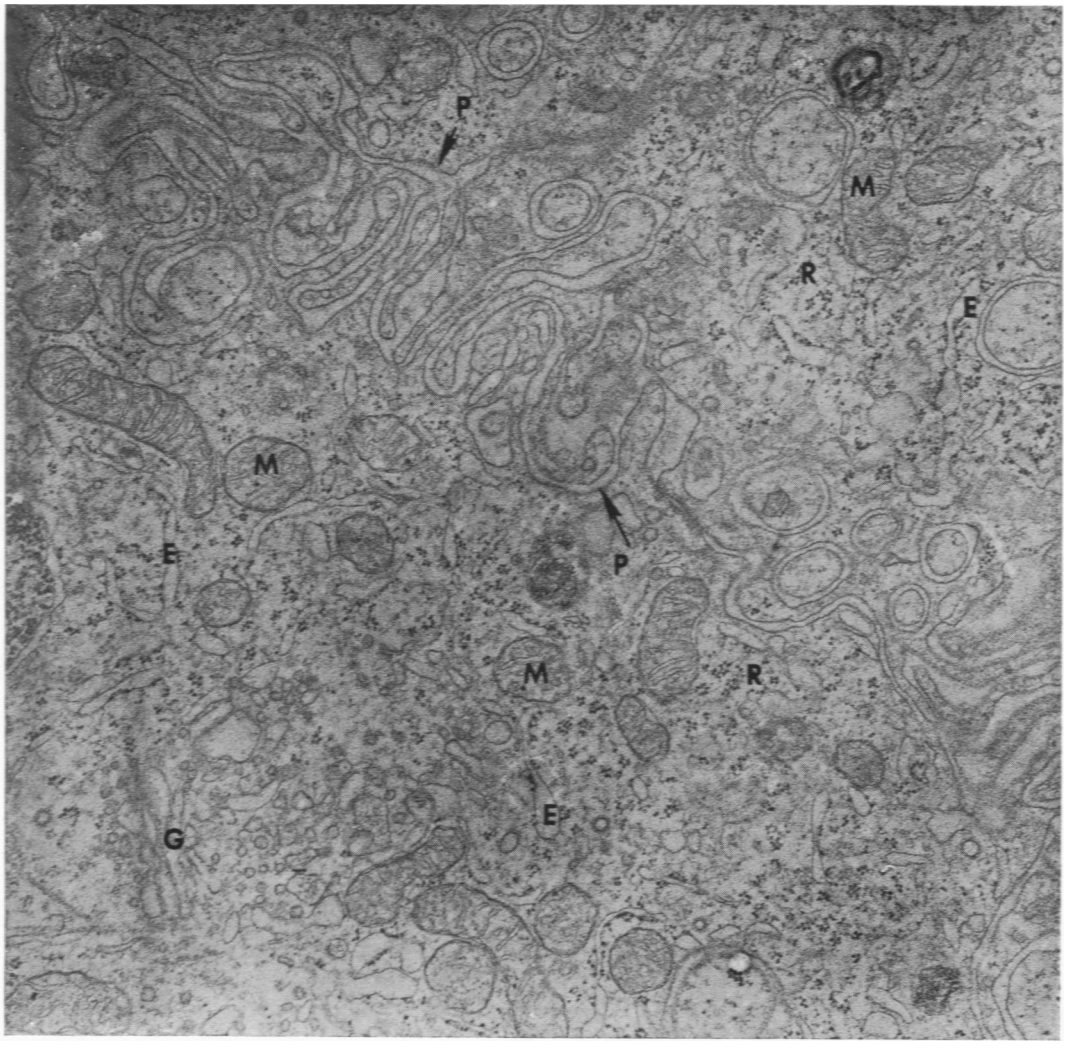

Fig. 2. Two cells of an "active" gland from Group IB. Golgi *(G)* apparatus in lower cell is prominent, with numerous vacuoles and vesicles. Dispersed sacs of granular endoplasmic reticulum *(E)* are numerous. Free ribosomes *(R)* are largely present in aggregates. Plasma membranes *(P)* of adjacent cells have numerous interdigitations. Granules are not prominent in the mitochondria *(M)*. × 25,600.

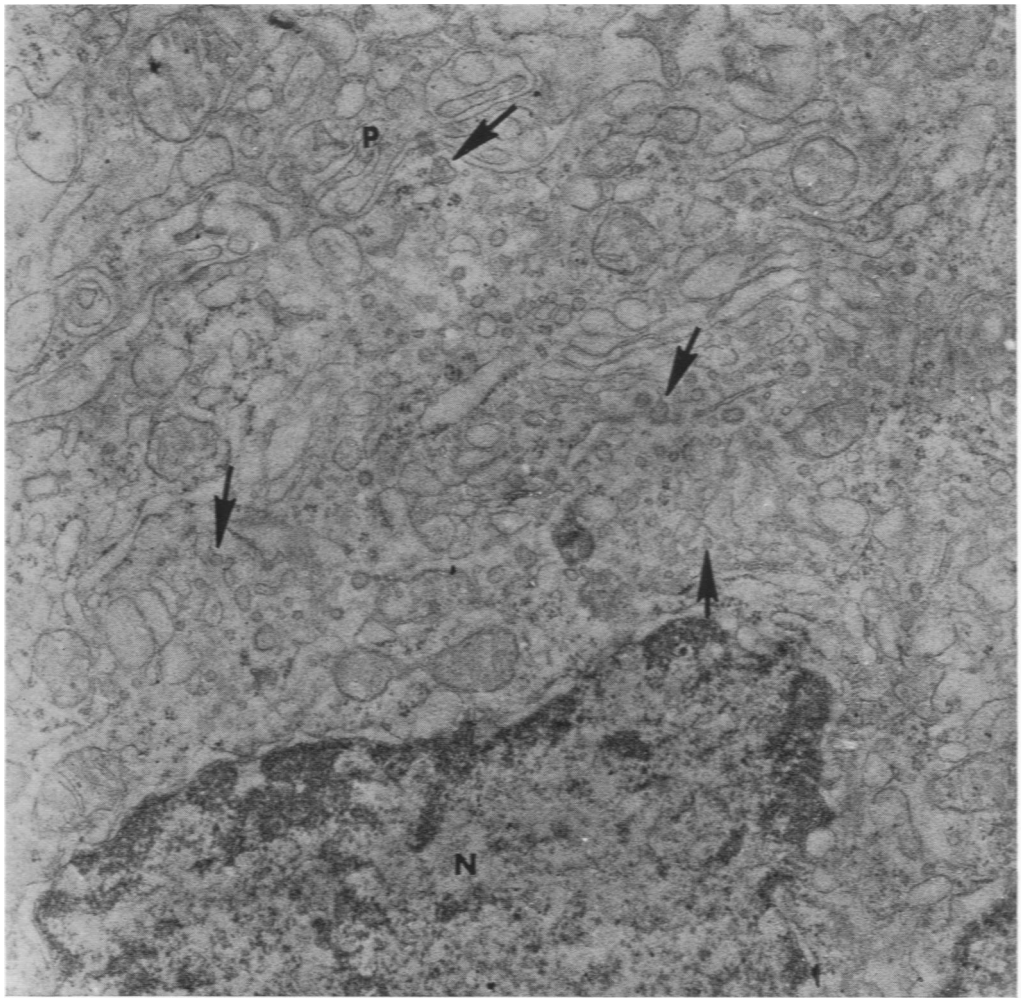

Fig. 3. Active chief cell from an "active" gland, Group IC. The Golgi apparatus contains numerous vacuoles with slightly electron-dense contents which have been called prosecretory granules *(arrows)*.[7] The plasma membrane *(P)* is tortuous. The nuclear *(N)* outline is irregular. × 25,600.

Fig. 4. "Normal" parathyroid gland from Group IIE. Plasma membranes are moderately tortuous. Golgi apparatuses *(G)* vary in size in different cells, the upper two being large with many vacuoles, while the lower two are small and relatively inconspicuous. One array of granular endoplasmic reticulum *(E)* is present. A multivesicular body *(V)* is present, but there are no secretory granules. Small granules are present in almost every mitochondrion *(M)*. The nucleus *(N)* is regular in outline. × 25,600.

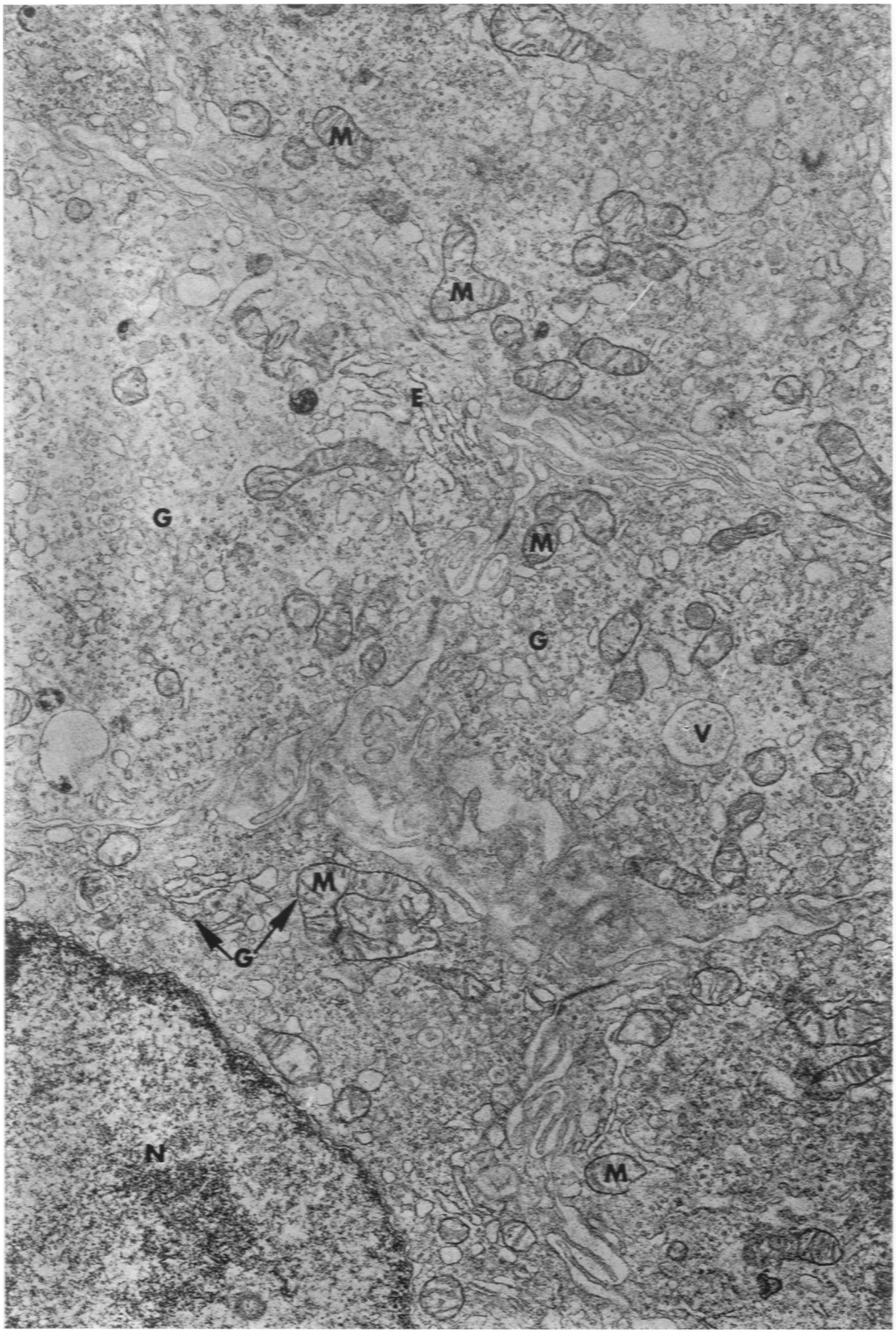

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Fig. 5. Normal parathyroid gland from control animal of Group IIA. The ultrastructure is identical to that shown in Fig. 4 for an animal from Group IIE. The plasma membranes are moderately tortuous. There is marked variation in Golgi size from large ones *(G)* with prominent vesicles and vacuoles to small ones *(arrows)* with few vacuoles and vesicles. No secretory granules are present. Some mitochondria *(M)* contain granules. Nuclei *(N)*; multivesicular body *(V)*; centriole *(C)*. Desmosomes *(D)* are present along the plasma membranes *(P)*. × 11,000.

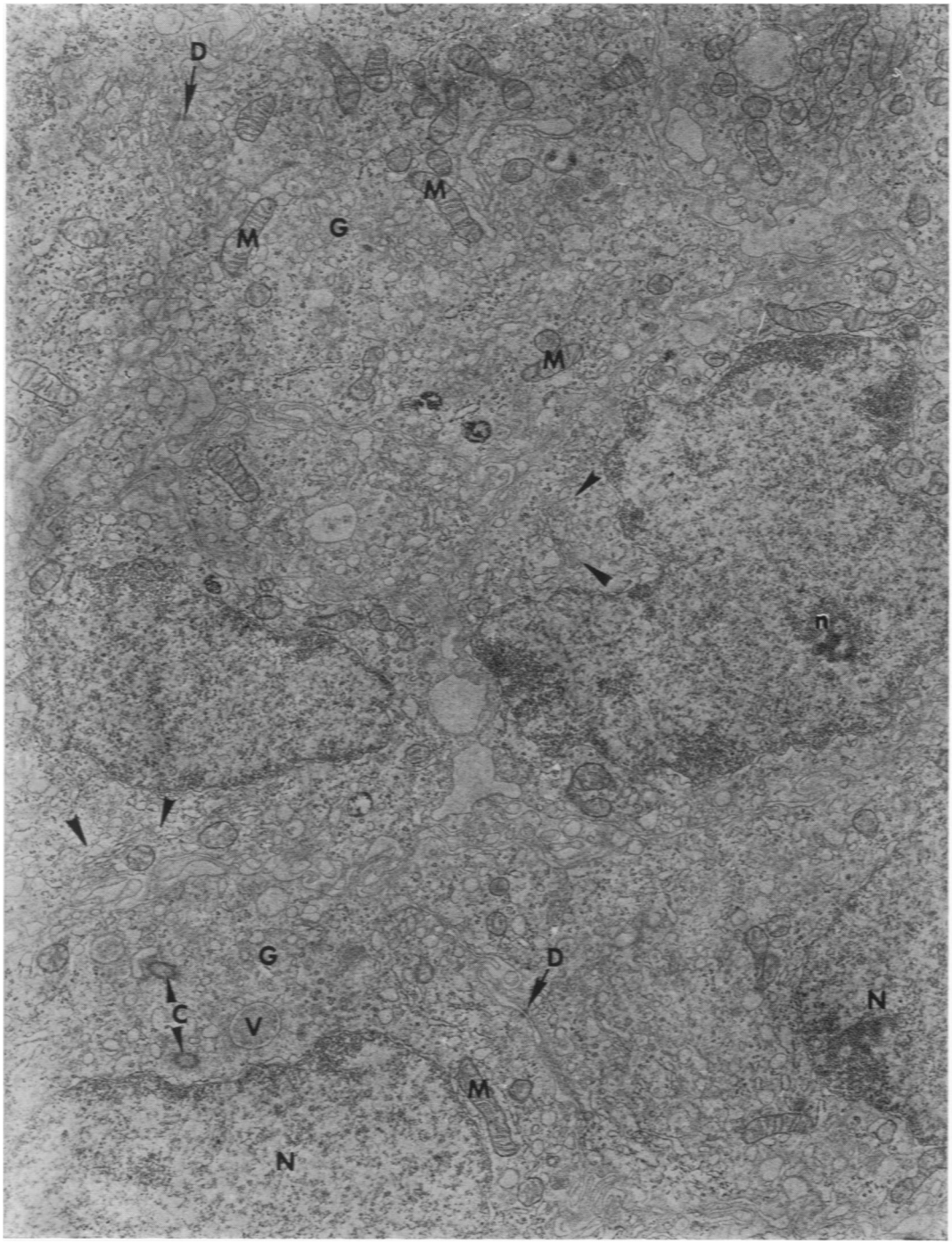

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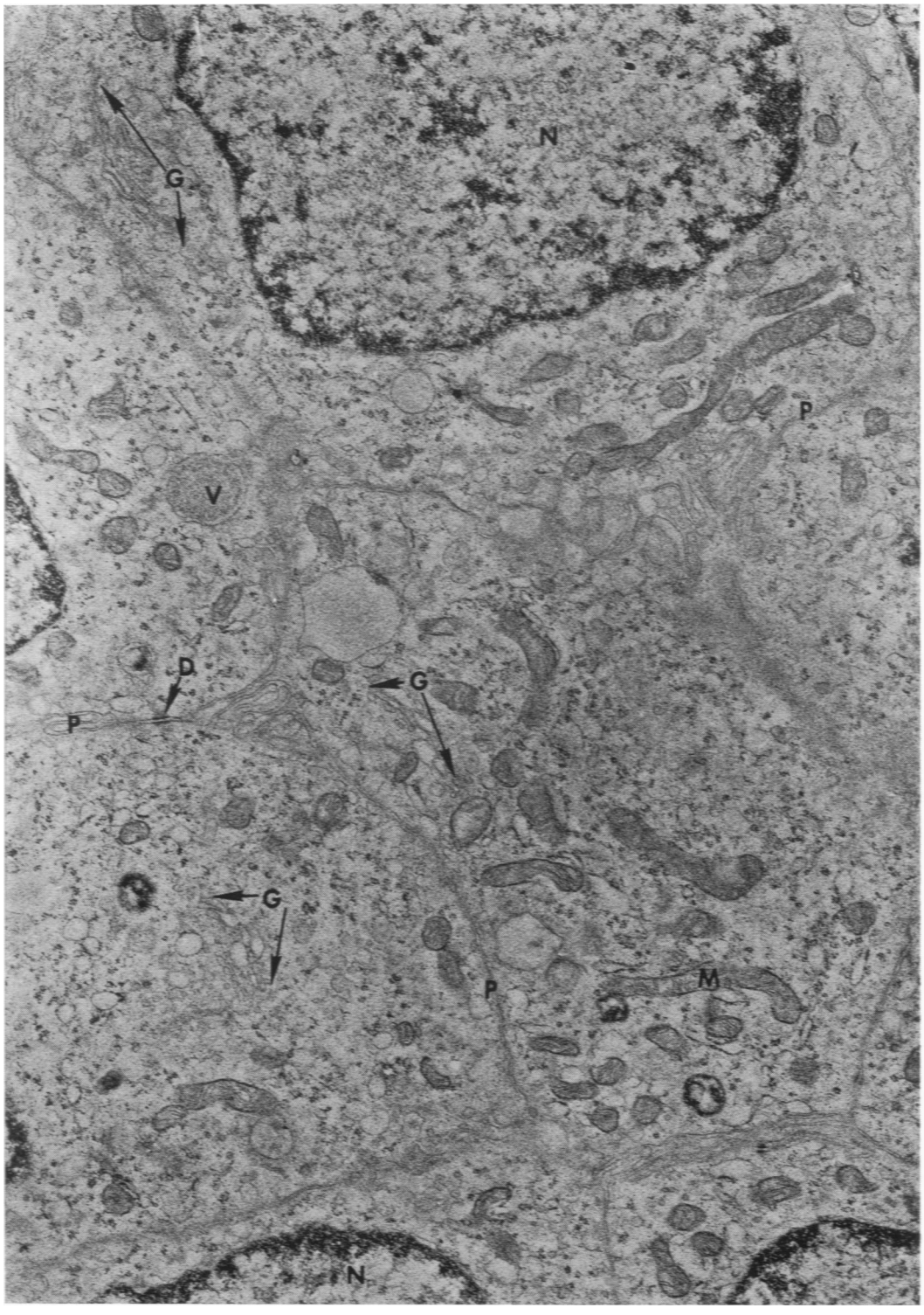
Fig. 6. Chief cells from "normal" gland, Group IIC. Three of the cells have prominent arrays of granular endoplasmic reticulum *(E)*, while one has a large multibranched Golgi apparatus *(G)* with numerous vacuoles and vesicles. × 10,700.

Fig. 7. Suppressed gland, Group IID. Chief cells are small with relatively straight and parallel plasma membranes. Granular endoplasmic reticulum *(E)* is dispersed in all cells. Golgi apparatuses *(G)* are small with few vacuoles and vesicles. Most mitochondria *(M)* are free of granules. Multivesicular bodies *(V)*; nuclei *(N)*. Desmosomes *(D)* are present along the plasma membranes *(P)*. × 14,500.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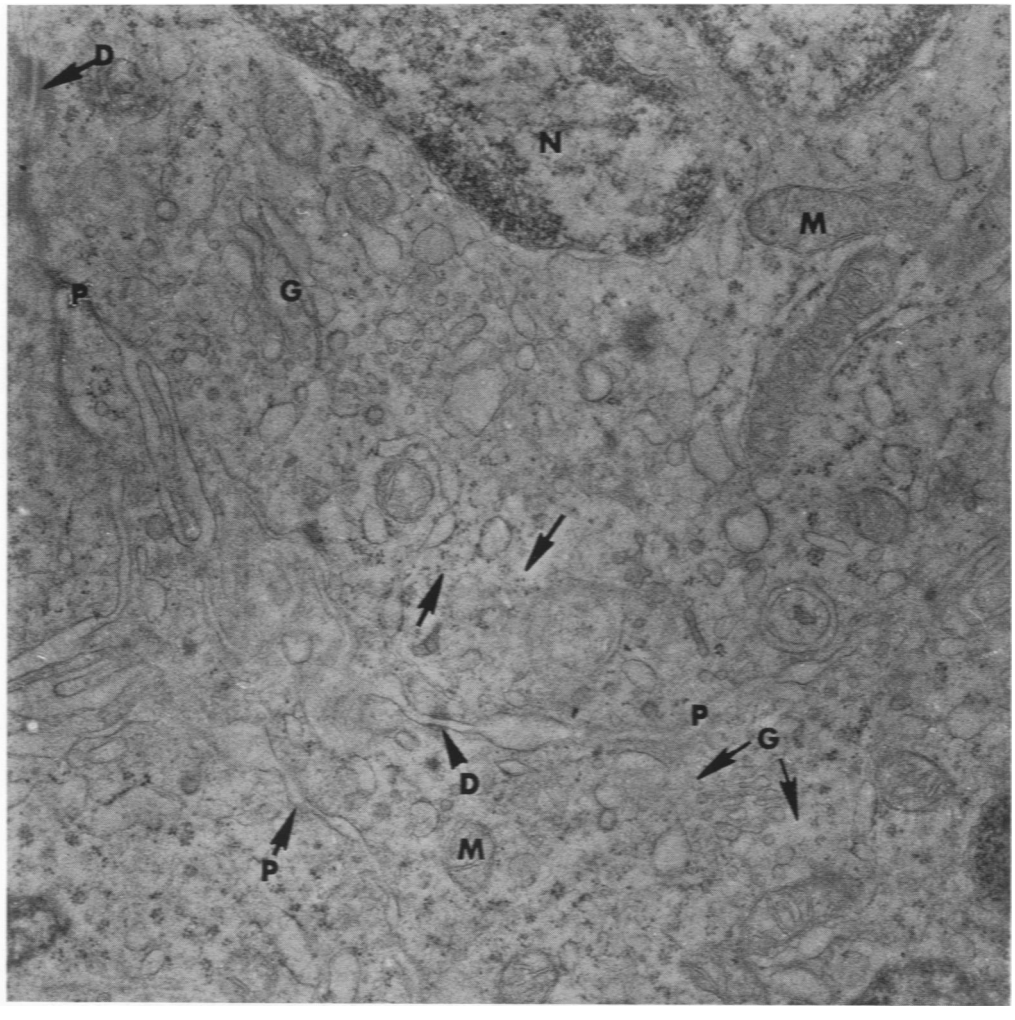

Fig. 8. Suppressed gland, Group IC (Table 1), showing relatively straight plasma membranes *(P)*, small Golgi apparatuses *(G)* with few vacuoles and vesicles, and a moderate number of dispersed ribonucleoprotein particles *(arrows)*. Nuclei *(N)*. × 25,600.