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The effects of long-term low-protein intake on gastrin cells of the rat antral mucosa during adulthood

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Summary. The effect of experimental protein malnutrition on gastrin producing cells in the antral part of the stomach was studied in male Wistar rats. Isoenergetic diets containing 25% (C-25) or 6% (PD-6) were given in isocaloric amounts during a 4-month experiment. All rats were offered drinking water ad libitum. The results showed that the long-term protein diet did not produce changes in the gastrin cell number. At the ultrastructural level G cells exhibited a decreased size of the nucleus. They were found to have an increased total granule volume density but the volume density of dense-cored granules was lower. The serum gastrin levels were significantly lowered by feeding the low protein diet. These changes are compatible with decreased functional activity of G cells under long-term protein deprivation.

Key words: G cell, Morphometry, Rat, Hypoprotein nutrition

Introduction

Different types of endocrine cells have been found in the antral part of the stomach, among which the most numerous are gastrin-producing cells. There are a number of reports about number and ultrastructural appearance of gastrin cells (G cells) in a variety of pathological and experimental conditions. These conditions include pernicious anaemia (Polak et al., 1971a), acromegaly (Creutzfeldt et al., 1971), duodenal ulcer (Creutzfeldt et al., 1976), vagal denervation, portocaval shunting (Alumets et al., 1980), hyperparathyroidism (Polak et al., 1971b), antral exclusion (Alumets et al., 1979) and hypophysectomy (Bastie et al., 1988). Changes in the gastrin levels, number and appearance of G cells have also been described during a feeding-fasting cycle (Lichtenberger et al., 1975, 1976; Bertrand and Willems, 1980).

It is well known that the physiological functions of a majority of the organs are compromised to some extent during protein malnutrition (Alleyne, 1966, 1967; Ahmad and Rahman, 1975; Edozien and Switzer, 1978). Probably the most significant changes are in the gastrointestinal tract (Hopper et al., 1972; Shrader et al., 1977; Hatch et al., 1979). Besides the interest which has been focused on the endocrine cells of the islets of Langerhans (Volk and Lazarus, 1960; Platt and Stewart, 1967; Dixit and Kaung, 1985) during protein malnutrition, there have been no experimental studies about the effects of a hypoprotein diet on the other gastrointestinal endocrine cells, especially relating structure to function in those cells.

The aim of this study was to examine the influence of long-term feeding of a low-protein diet on the number and ultrastructure of antral G cells and to correlate any changes with levels of serum immunoreactive gastrin (IRG).

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Materials and methods

Twenty-four Wistar rats, aged 2 months and weighing between 200-220 g at the beginning of the experiment, were individually housed in cages, in a temperature-controlled room with a 12-hour light-dark cycle and free access to water. Control rats (n= 12) were fed a 25% protein diet (C-25) and protein-deprived rats (n= 12) a 6% protein diet (PD-6). The composition of the diets is given in Table 1. The two diets were isoenergetic and complete with respect to carbohydrate, fat, vitamin and mineral content. Both of the diets were given in isocaloric amounts (380 kJ/day) during the 4 months the experiment lasted.

After 4 months on one of the two diets, the rats from both groups were starved for 24 hours with water available ad libitum before being killed in the morning by a blow on the neck.

Measurement of gastrin concentration in serum was performed by radioimmunoassay (GASTRIN RIA KIT «CIS») in 9 rats from each experimental group.

For histological examination tissue samples from the antral portion of the stomach were fixed in Bouin's solution for 12 hours, dehydrated by routine methods

and embedded in paraffin (Pearse, 1980). Gastrin cells were stained employing an immunoenzyme PAP method according to Stenberger (1986), using anti-gastrin serum (diluted 1:800; a kind gift from Dr. Rehfeld). The results were analyzed on 5 μm-thick sections. Only sections cut perpendicular to the mucosal surface were taken into account. The frequency of stained cells was calculated by counting the number of cells per visual field using a x40 objective and x10 eyepiece. Cell counts were made on five randomly selected visual fields on one section from each rat.

For electron microscopy tissue samples were taken from four rats for each group. Small blocks of antral mucosa were cut and immersed in 3% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4) for 3 hours. All specimens were post-fixed for 1 hour in 1% osmium-tetroxide in the same buffer, dehydrated in a series of ethanol concentrations and propylene oxide, and embedded in Epon 812. Ultrathin sections were cut on an LKB Ultratome, double stained with uranyl acetate and lead citrate under the same conditions, and examined in an Opton 109 electron microscope. Longitudinal sections of antral mucosa were examined for G cells in a random manner (Weibel et al., 1969). The material from each group was pooled. G cells were identified by their well-defined ultrastructure (Solcia et al., 1975). Their most characteristic feature was the secretory granule population they contained. Forty-four G cells from control and forty-seven G cells from the experimental group were examined morphometrically. The cells were used for morphometry only if sectioned in such a way that the nucleus was visible. Micrographs were prepared at constant magnification (x 12,000).

Morphometric analysis was performed by covering the test micrographs with a transparent double lattice point-counting grid using the methods of Weibel (Weibel et al., 1969). The test grids were used to obtain «hits» on test organelles of interest (coarse points for nucleus and cytoplasm, and fine for the granules). The following morphometric parameters were obtained from these prints: for the whole cell, the profile area, for the nucleus, the profile area, volume density, and for the granules, volume density, number of profiles per section, and number of profiles per mm^2 of cytoplasm. Granules were divided into two types: 1) «dense cored», and 2) all others that were «pale» with either a homogeneous core of lower electron density or an eccentrically placed core surrounded by an electron-lucent halo, and «empty» granules with a very electron-lucent interior. The

Table 1. Composition of diets.

INGREDIENTS (g/100g)	CONTROL	PROTEIN DEFICIENT
Dried skimmed milk (34% protein	7.50	3.00
Fish meal (70% protein)	16.00	2.00
Soyabean meal (42% protein)	10.00	0.50
Alfalfa meal (17% protein)	15.00	16.00
Gluten (63% protein)	3.00	0.10
Cornstarch (8% protein)	3.50	1.00
Wheat meal (10% protein)	2.00	1.00
Bran (15% protein)	5.00	2.00
Yeast (48% protein)	2.00	0.10
Sucrose	11.10	11.10
Starch	19.90	56.40
Corn oil	2.50	4.30
Vitamin-mineral mixture[1]	2.25	2.25
Energy density kJ/g	15.93	16.64
Calculated proximate composition[2]		
Crude protein	24.58%	5.94%
Crude fat	4.83%	4.94%
Crude carbohydrate	57.27%	79.84%
Crude fober	5.38%	4.33%
Ash	5.44%	2.45%

[1]: supplied per kg diet: vitamin A, 6050 IU; vitamin D, 5060 IU; vitamin K, 3.1 mg; alfatocopherol acetate, 22 IU; choline, 600 mg; pholic acid, 2.4 mg; niacin, 33 mg; alfa-phantothenic acid, 20 mg; riboflavin, 3.7 mg; thianine, 11 mg; vitamin B12, 4.4 mg; pyridoxine, 1.9 mg; biotin, 0.15 mg; $CaCo_3$, 0.50 g; $CaHPO_4$, 1.25 g; NaCl, 0.50 g; $CaCl_2.6H_2O$, 1.77 mg; $CuSO_4.5H_2O$, 17.28 mg; $FeSO_4.7H_2O$, 673.32 mg; $MnCl_2.4H_2O$, 237.71 mg; $ZnSO_4.7H_2O$, 79.16 mg; KJ, 2.00 mg. [2]: feed formulation prepared by Agricultural Reseaarch Center, Charles Pfizer and Co. inc., Terre Haute, IN. All components of the diets were obtained from Veterinarski Zavod, Batajnicki drum 4, 11080 Zemun, Yugoslavia.

Table 2. Ultrastructural properties of antral G cells in the control (C-25) and experimental (PD-6) groups of rats.

MORPHOMETRIC PARAMETERS (X±SEM)	C-25	PD-6	STATISTICAL SIGNIFICANCE (p<)
Cell			
No. of cells analysed	44	47	
Profile area (μm^2)	61.68±1.71	56.09±2.07	0.05
Nucleus			
Profile area (μm^2)	23.68±1.09	19.65±1.12	0.05
Volume density (% of cell area)	38.55±1.49	35.51±1.74	0.05
Cytoplasm			
Profile area (μm^2)	38.00±1.40	36.44±1.76	n.s.
Cytoplasmic granules			
Volume density (% of cytoplasm)	19.61±1.09	28.11±1.07	0.001
Dense cored granule volume density	4.37±0.28	2.70±0.15	0.001
Vol. density of all other granules	15.24±1.08	25.41±1.07	0.001
No. of granules per cell profile	113.73±7.84	125.87±8.68	n.s.
No. of granules per mm^2 cytoplasm	3.04±0.20	3.49±0.22	n.s.

proportion of «dense cored» granules was recorded for each micrograph.

The diameter of all granule profiles was measured in 9 cells from each experimental group. The mean granule radius was measured using a Kotron MOP AMO3 semiautomatic analyzer. Test micrographs were placed on the measuring tablet and a light-weight cursor was used to mark two radii of each granule.

All results were expressed as the mean ± SEM. Statistical analysis was made with student's test; p values of 0.05 were regarded as statistically significant.

Results

There was no significant difference between the groups in the final mean body weight (376.1±6.7 vs. 389.2±6.6g).

Gastrin cells were numerous in the antral part of the stomach and occupied mainly the basal part of the glandular area. Following long-term consumption of the hypoprotein diet there was no change in the gastrin cell number compared with controls that were fed with a diet containing the optimal amount of protein (Fig. 1).

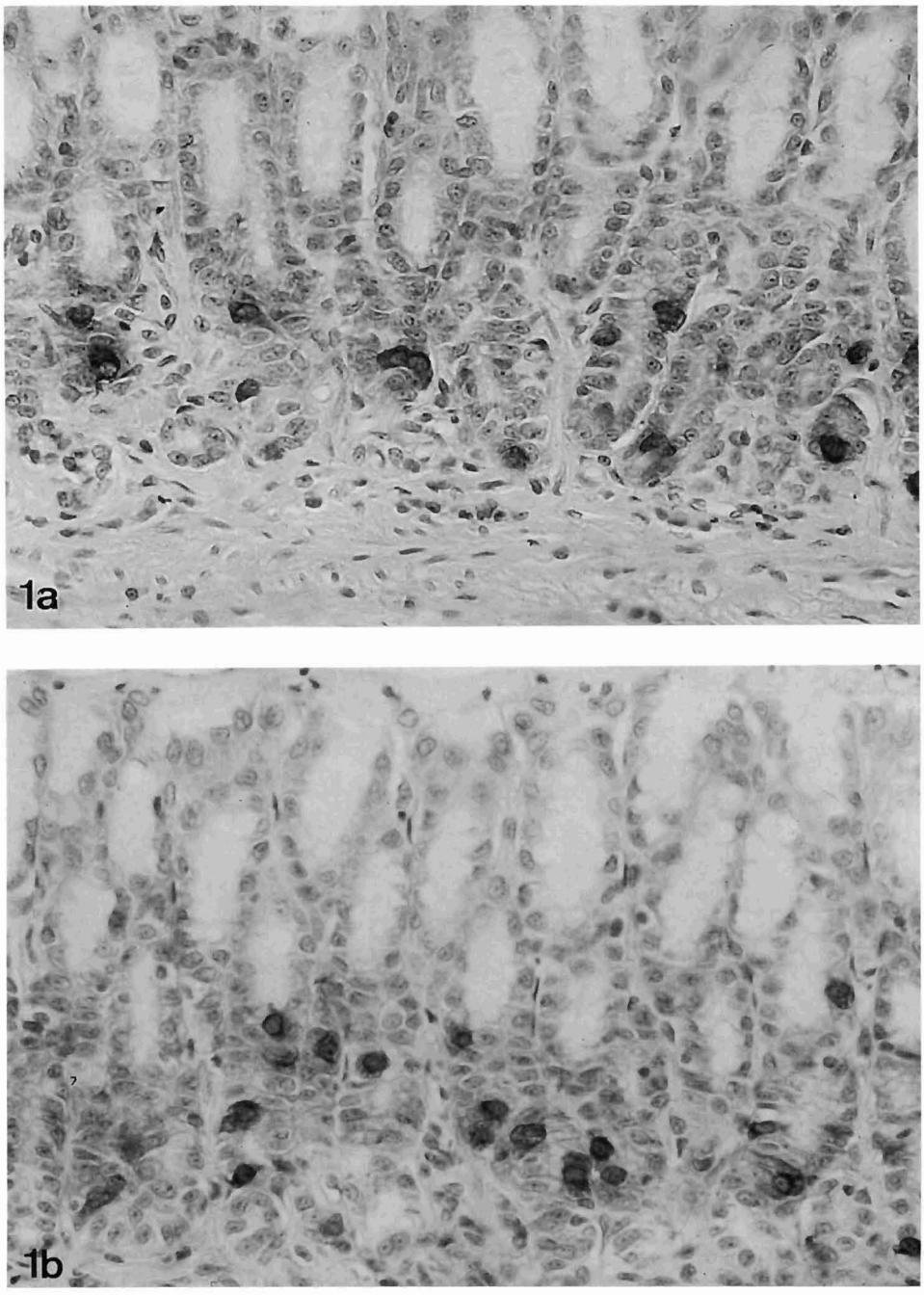

Fig. 1. Pyloric gland area of control **(a)** and protein-deprived rat **(b)**. Gastrin immunoperoxidase (PAP) staining. There is no change in the gastrin cell number between the groups. x 500

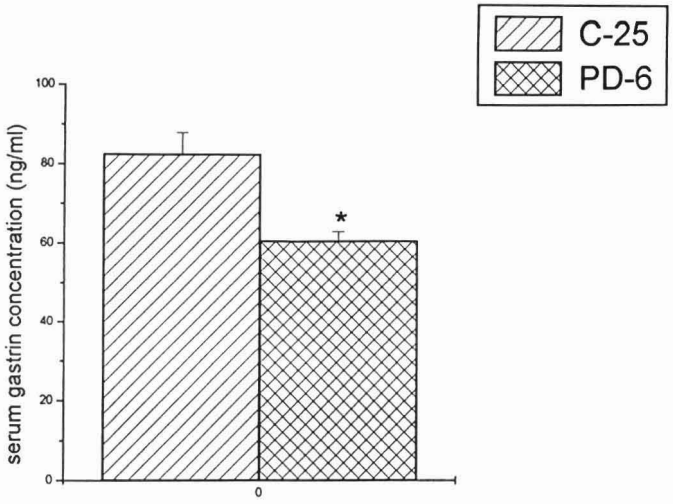

Fig. 2. Serum gastrin concentration in control (C-25) and protein-deprived (PD-26) rats. *: $p<0.05$.

The serum gastrin levels were significantly lowered by feeding the low-protein diet (82.56±5.37 vs. 60.53±2.43 ng/ml) (Fig. 2).

The results of morphometric measurements are summarized in Table 2. Over the four-month period of low-protein intake a significant decrease in cell profile area occurred which reflected a slight decrease in cytoplasm profile area and a significant decrease in profile area of the nucleus. It was found that the G cells in the experimental group had an increased granule volume density because of the increased volume density of empty and pale granules. The volume density of dense cored granules was decreased in the G cells of malnourished rats. Dense cored granules were also less numerous in the gastrin cells of the experimental group (Figs. 3, 4). This was reflected in a smaller proportion of dense cored granules in G cells of the malnourished rats than in the control rats: 8.0±1.4% of the G cell granules in the malnourished rats were dense cored compared to 17.9+2.1% in the controls. There was no difference in

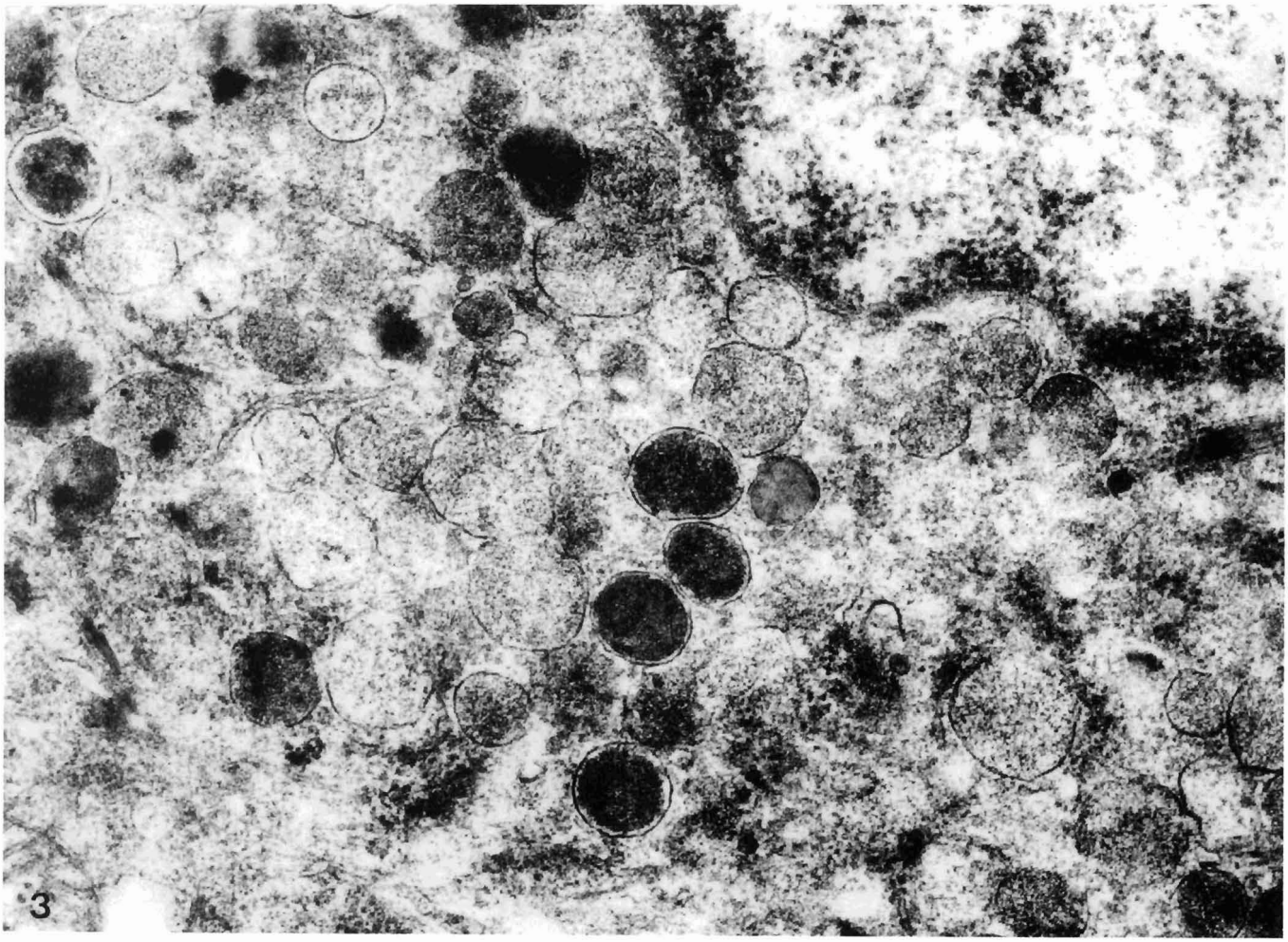

Fig. 3. Electorn micrograph of gastrin cell from the pyloric gland area of control rat. Normal ultrastructure is characterized by the presence of secretory granules of different electron density. Uranyl acetate, lead citrate. x 34,800

the total number of granules per unit of cytoplasm. The granule profile diameter in the G cells of the experimental group was increased (Table 3).

Discussion

Besides an adequate energy intake, an optimal amount of protein in the diet is necessary for the normal function and integrity of cells. The mean daily energy intake of rat weighing 300 g is about 320 kJ with 15-25% originating from protein (Nadeli and Desai, 1981).

Table 3. Mean granule profile diameter (nm) in gastrin cells.

	GRANULE PROFILE DIAMETER (X±SEM)
C-25	255±7.5 (n=1146)
PD-6	293±7.6 (n=1498)
Statistical significance	p<0.001

n: number of analyzed granules.

Our rats were pair-fed (380 kJ/day) because we wished to isolate the effects of protein malnutrition. We did not find a significant difference between the experimental and control groups concerning final body weight and we conclude that the normal body mass gain in protein-deprived animals was provided by the adequate daily energy intake. The results are in accordance with the observation of Le for protein depleted rats during their adulthood (Le, 1983).

The results of the histological investigation showed that long-term hypoprotein nutrition did not affect the number of gastrin-producing cells per visual field.

At the ultrastructural level, antral G cells were identified by the secretory granules they contained. Many investigators think that the electron density of secretory granules reflects the age of the granules and the processing of synthesized gastrin. Golgi-associated, newly-formed, immature granules which contain the proform of gastrin (G-34) generally displayed a higher electron density than more peripherally located (older)

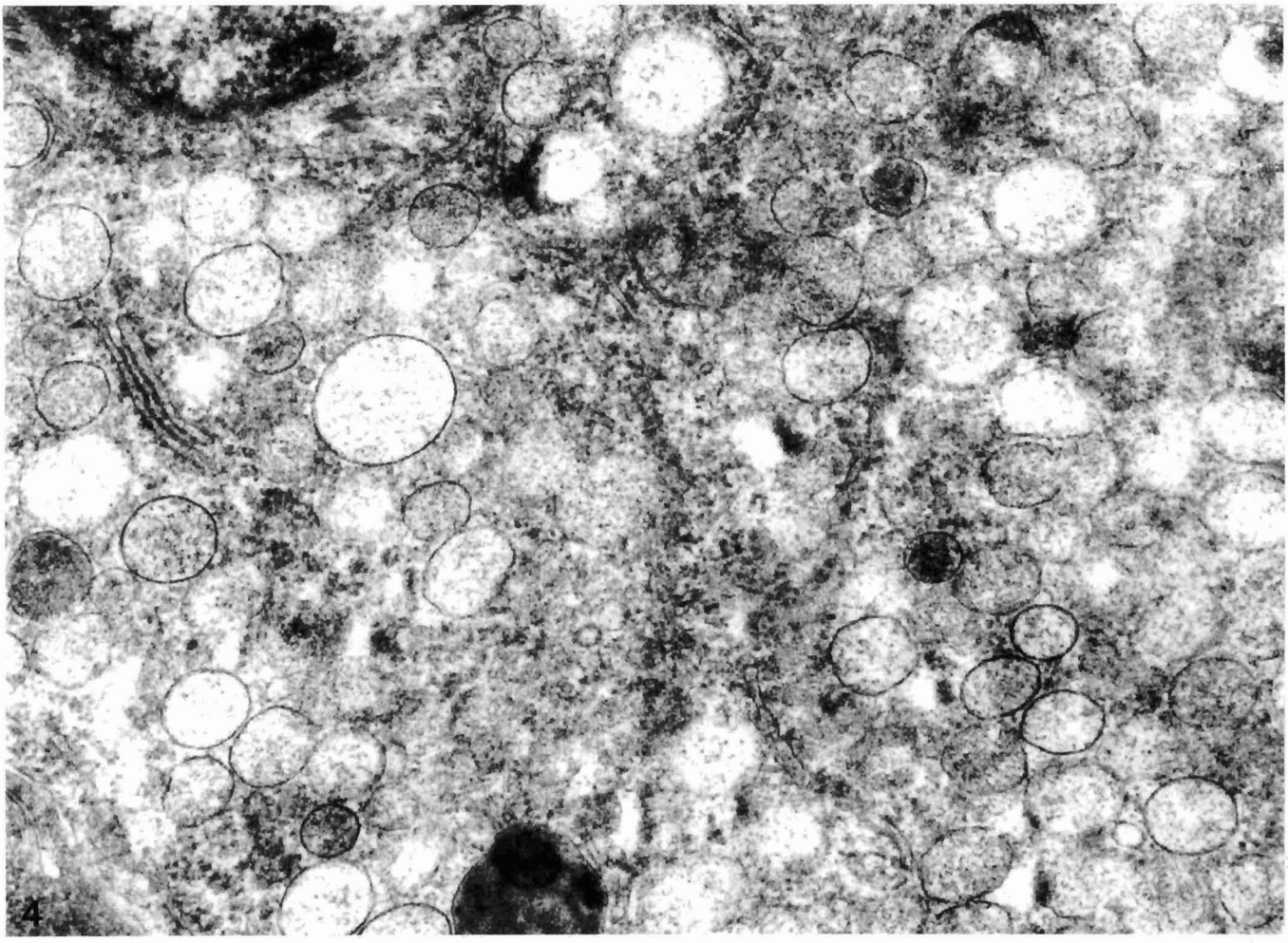

Fig. 4. Electron micrograph of gastrin cell from the protein-deprived rat. Note that dense core granules are less numerous in the gastrin cell from this group Uranyl acetate, lead citrate. x 34,800

granules which contained gastrin G-17 (Rahier et al., 1987). Alumets found the majority of the secretory granules in the antral gastrin cells in the fasted rats to be electron lucent (Alumets et al., 1979). Because our rats were fasted overnight, the appearance of G cells from our experiment agreed with this statement. The results obtained for the morphometric analysis showed that the antral G cells from the protein-deprived rats exhibited increased granule volume density (i.e. the proportion of cytoplasm occupied by granules). Dense cored granule volume density was decreased whereas volume density of all other granules was increased. It was also found that the proportion of dense cored granules was lower in the G cells of the malnourished rats. Together with a decrease in the size of the nucleus (a classic index of functional activity, Hellman and Hellestrom, 1959) we interpret these changes as a sign of decreased synthesis in the G cells obtained from the protein depleted rats. If the release of granules was normal, granule volume density would have been decreased in those G cells, but we found that the granule volume density was increased predominantly by the increase of the volume density of pale and empty granules. It seems that both synthesis and release appear to be inhibited. Up to now there have been no data about the effects of long-term hypoprotein nutrition on the morphofunctional state of these cells. Similar indications of decreased secretory function (a great proportion of pale granules) were obtained in ß cells of rat pancreas during one month feeding with a diet containing a low level of protein (Weinkove et al., 1977). The increase of the mean granule profile diameter in the gastrin cells from the experimental group can be explained by predominance of the older granules with bigger diameter compared with younger ones in the whole population of secretory granules.

Our morphometric data concerning the effects of chronic protein depletion agree with the findings of decreased serum immunoreactive gastrin in those animals and indicate that this experimental condition resulted in a decreased morphofunctional activity of G cells.

There are numerous data about humoral and paracrine interactions among the endocrine cells (Chiba et al., 1980a,b). Thus, there is a well-known inhibitory effect of somatostatin on the release of gastrin from the gastrin cells (Koop et al., 1988; Seefried et al., 1988). Younoszai and Dixit (1980) showed that the release of insulin from ß cells was inhibited by an increased content of somatostatin in rat pancreas tissue after 12 weeks' treatment with a 4% casein diet. Koko et al. (1989) found the number of somatostatin producing cells was significantly increased both in the oxyntic and pyloric mucosa of protein-depleted rats. The number of D cells was increased especially in the pyloric region. Maybe this augmentation could explain the changes obtained for the gastrin cells in our experimental conditions. Unfortunately, there are no data about the effects of a hypoprotein diet on the morphofunctional status of D cells. Further investigations are required in order to verify the hypothesis described above.

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