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Ultrastructure of the ventromedial hypothalamic nucleus in fasted and refed young and old rats

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Many hypothalamic nuclei are involved in the regulation of food intake and energy homeostasis. An ultrastructural investigation of the hypothalamic ventromedial nucleus (VMN), a hypothetical "satiety centre" was performed to explore the morphological basis of altered feeding behaviour of old rats in an experimental model of fasting/refeeding. Young (5 months old, n = 12) and old (24 months old, n = 12) male Wistar rats were fasted for 48 hours, then refed for 24 hours and sampled thereafter. Brain tissue was fixed by perfusion, histological and ultrathin sections were obtained by routine methods. Although food intake was similar in control young and old rats, during refeeding old animals consumed less chow than young ones. The EM analysis of VMN neurones of old control rats revealed, besides typical age-related residual bodies, deep indentations of the nuclear envelope and the presence of long, undulating rough endoplasmic reticulum cisternae in the cell periphery. In both young and old rats fasting for 48 hours led to the expansion of Golgi complexes and increased folds of the nuclear envelope, which is suggestive of enhanced cellular activity of the VMN neurones. These fasting-induced alterations were sustained in the VMN neurones of refed rats in both age groups. The results showed that the VMN neurones of old control rats differ at the ultrastructural level from young ones. However, starvation and subsequent refeeding cause similar alterations in the hypothalamic neurones of "satiety centre" of both young and old rats.

Key words: ageing, ventromedial nucleus, electron microscopy, fasting, refeeding

INTRODUCTION

The process of ageing diminishes the capability of organisms to adapt to the changes of the internal and external environment. The deterioration of homeostatic mechanisms in ageing becomes often apparent upon physiological or pathological stimulation. Alterations in the nutritional state, whether short-term or chronic, profoundly affect various aspects of metabolic and hormonal homeostasis [5]. Fasting and subsequent refeeding belong to nutritional manipulations which reflect naturally occurring periodicity in nutrient supply. We have previously demonstrated that old rats that were refed after fasting for 48 hours, showed significant changes, as

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compared to young animals, in the rate of liver glycogen resynthesis [13], structure of thyroid gland [8, 10] and serum concentrations of thyroxine, triiodothyronin, parathormone and calcitonin [8, 9]. Moreover, during the refeeding experiments we noticed that the food intake of refed old rats was lower than that of young animals; this effect was also found by other authors in middle-aged rats [7]. Hypothalamic nuclei, such as lateral hypothalamic area (LHA, "hunger centre") and ventromedial hypothalamic nucleus (VMN, "satiety centre"), as well as arcuate and paraventricular nuclei were shown to control food intake, energy homeostasis and body mass [4, 6]. In an attempt to elucidate the mechanisms of the ageing-related down-regulation of food intake during refeeding we decided to characterise first the ultrastructure of neurones of the ventromedial hypothalamic nucleus of young and old rats that were fasted for 2 days and then refed for 1 day.

MATERIAL AND METHODS

Animals

Inbred male Wistar rats aged 5 months (young, 338 ± 22 g, n = 12, mean ±SD), and 24 months (old, 483 ± 33 g, n = 12) were used. The mean and the maximal life spans of this rat colony were 26 and 32 months, respectively. The animals were housed 2 or 3 per cage and were maintained on a controlled light schedule (light on 7:00–19:00) at 20 ± 1°C. They were fed a standard diet containing (w/w) 13% protein, 55.5% carbohydrate, 2.5% lipid, 1% calcium, 0.75% phosphates and 27% indigestible compounds (Labofeed B, Kcynia, Poland). The care and treatment of the animals were in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes". The study was approved by the Local Ethical Committee for Animal Experiments, Gdańsk, Poland.

Dietary manipulation and sampling

Control rats (Group I) were fed *ad libitum* and sampled correspondingly at the same time of the day as fasted and refed animals. Other animals were fasted starting from 8:00 and sampled after 48 hours (Group II), or fasted for 48 hours, provided with food at 8:00 (start of refeeding) and sampled 24 hours thereafter (Group III). Food consumption was measured for 2 or 3 rats present in one cage and changes in body weight were determined for each rat. The body weight of young rats in groups I, II and III was 342 ± 27 g (mean ±SD, n = 4 in each group) 302 ± 21 g, and 338 ± 17 g, respectively. The body weight of old rats in groups I, II and III was 485 ± 43 g, 448 ± 44 g, and 468 ± 37 g, respectively. Young and old rats of Group I consumed over 24 hours 10.8 ± 1.1 g, and 9.3 ± 1.3 g chow per 100 g b.w., respectively. After 24 hours of refeeding, the average food consumption of young and old rats was 13.8 ± 1.1 g and 9.5 ± 0.8 g of chow per 100 g b.w., respectively.

Sample preparation

Rats were anaesthetised by the intraperitoneal injection of pentobarbital sodium (50 mg/kg b.w.). The animals were preperfused transcardially with 50 ml of 0.9% saline containing 500 heparin units, and then perfused with a fixative composed of 4% paraformaldehyde and 1% glutaraldehyde in 0.05 M phosphate buffer (pH 7.4) containing 0.03% $CaCl_2$. Brains were rapidly removed from the skulls, and post-fixed in the same fixative overnight at 4°C. Next, thick tissue blocks that contained the whole rostrocaudal extent of the hypothalamus were dissected, and from the blocks that contained middle tuberal hypothalamus 500 μm coronal serial slices were cut with vibratome 1000 S (Leica, Germany). From tissue slices one specimen was taken with a needle of 1 mm diameter that was driven unilaterally (left side) into ventromedial hypothalamic nuclei. Each specimen was transferred directly into the fixative for 12 h, briefly rinsed in 0.1 M phosphate buffer containing $CaCl_2$ at room temperature, and then post-fixed for 1 hour in 1% osmium tetroxide. After dehydration in ascending concentrations of alcohols and propylene oxide the specimens were embedded in Epon 812. The description of the ultramicroscopic features of the VMN neurones was based on the analysis of 804 photographs of the hypothalami neurones of 24 rats.

To confirm that the specimens had been taken from the appropriate brain area, the slices with remaining brain tissue were fixed in 10% formalin for 2 days and embedded in paraffin. Microtome sections (20 μm) were stained with haematoxylin and eosin. Semithin Epon sections (1–2 μm thick) were cut from each block with Reichert Om U3 ultramicrotome, placed on glass slides and stained with 0.1% toluidine blue. By comparing under the light microscope semithin sections of the unilateral side with histological sections of the contralateral one, it was possible to eliminate the incorrectly taken specimens. Once the VMN was identified, the Epon blocks were trimmed to adequate sizes for electron microscopic

studies. The ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEM 1200EX II electron microscope.

RESULTS

VMN was located ventromedially to the fornix in the midtuberal hypothalamus on both sides of the third ventricle. Anterior, ventrolateral and dorsomedial subdivisions of the VMA are recognised [16]. We performed an ultrastructural study on the middle part of VMN, which in histological sections appeared as prominent oval cell mass. Only one nucleolus was observed in all analysed VMN neurones of both young and old rats.

Young animals

Group I. Generally, the VMN neurones of fed rats were spherical or oval in shape. A large centrally localised nucleus displayed evenly distributed pale chromatin. The prominent nucleolus was usually positioned slightly eccentrically and the nuclear envelope was moderately invaginated. The cytoplasm contained rough endoplasmic reticulum (RER) whose cisternae were arranged in stacks forming a typical Nissl body (Fig. 1). The perikaryon was abundant in free ribosomes and polyribosomes. Multiple Golgi complexes were located around the nucleus and consisted of sacs of varying lengths and widths. Mitochondria, in the shape of spheres or rods, were presumably localised in the perinuclear regions. Several lysosomes were also present (Fig. 1).

Group II. As compared to control rats, many VMN neurones of fasted animals showed prominent nucleolus that was adjacent to the nuclear envelope (Fig. 2.). The majority of neurones displayed extensive Golgi complexes localised typically in the perinuclear region. Golgi cisternae were surrounded by a large number of vesicles. In the majority of cells mitochondria were often localised peripherally in the cytoplasm. There were no differences detected in the appearance and amount of RER and ribosomes between neurones of control and fasted young rats.

Group III. Most of the VMN neurones of young refed rats resembled ultrastructurally those of control animals except for the localisation of RER and mitochondria at the cell periphery (Fig. 3). Impressive Golgi complexes could also be seen in the perikarya.

Old animals

Group I. In contrast to the VMN neurones of young rats, the majority of neurones of old animals showed highly invaginated nuclear envelope that formed relatively deep folds (Fig. 4). In some neurones the invaginations were deep enough to divide the nucleus into lobes. The prominent nucleoli were located frequently close to the nuclear envelope or its invagination. Different than in young rats, most of the VMN neurones of old animals contained RER in the form of long, undulating cisternae, which wound through the perikaryon and composed loosely organised Nissl body (Fig. 5). Numerous cytoplasmic inclusions probably represented lipofuscin granules and multivesicular residual bodies. Similarly to neurones of young fed rats the cytoplasm was abundant in free ribosomes and polyribosomes that were often dispersed between RER membranes.

Group II. Most of the VMN neurones of old fasted rats contained long, undulating RER cisternae that were localised rather peripherally than perinuclearly (Fig. 6). Abundant polyribosomes were randomly dispersed in the cytoplasm. Prominent Golgi complexes and numerous mitochondria were usually distributed in the perinuclear zone.

Group III. After refeeding most of the VMN neurones showed expanded RER cisternae (Fig. 7). Numerous Golgi complexes accompanied by vacuoles of different sizes showed more dilated sacks than in control rats. The mitochondria looked swollen. The nucleoli were observed often in the vicinity of the nuclear envelope or its invagination.

DISCUSSION

The ultrastructure of the VMN neurones of young or adult animals has been previously characterised [2, 11, 12, 16], however, there are only a few reports that describe the effect of ageing. In sexually immature rats Millhouse [12] distinguished two neurone types in VMN: he denoted smaller cells (99% of all VMN neurones) as type I, and bigger cells (1%) as type II neurones. In our material we have seen only oval and rounded smaller neurones that had the diameter of 12–18 μm. Similarly to other authors we observed in the VMN neurones of young rats a large nucleus with a prominent nucleolus, well-developed RER, numerous polyribosomes, conspicuous Golgi complexes and many Golgi-associated vesicles.

The ageing process did not significantly change the total number of VMN neurones in the rat [11, 14]. VMN neurones of control, both young and old, rats showed prominent Golgi complexes and cytoplasm abundant in free ribosomes and polyribosomes. In contrast to young animals we observed in the VMN neurones of old rats the presence of resid-

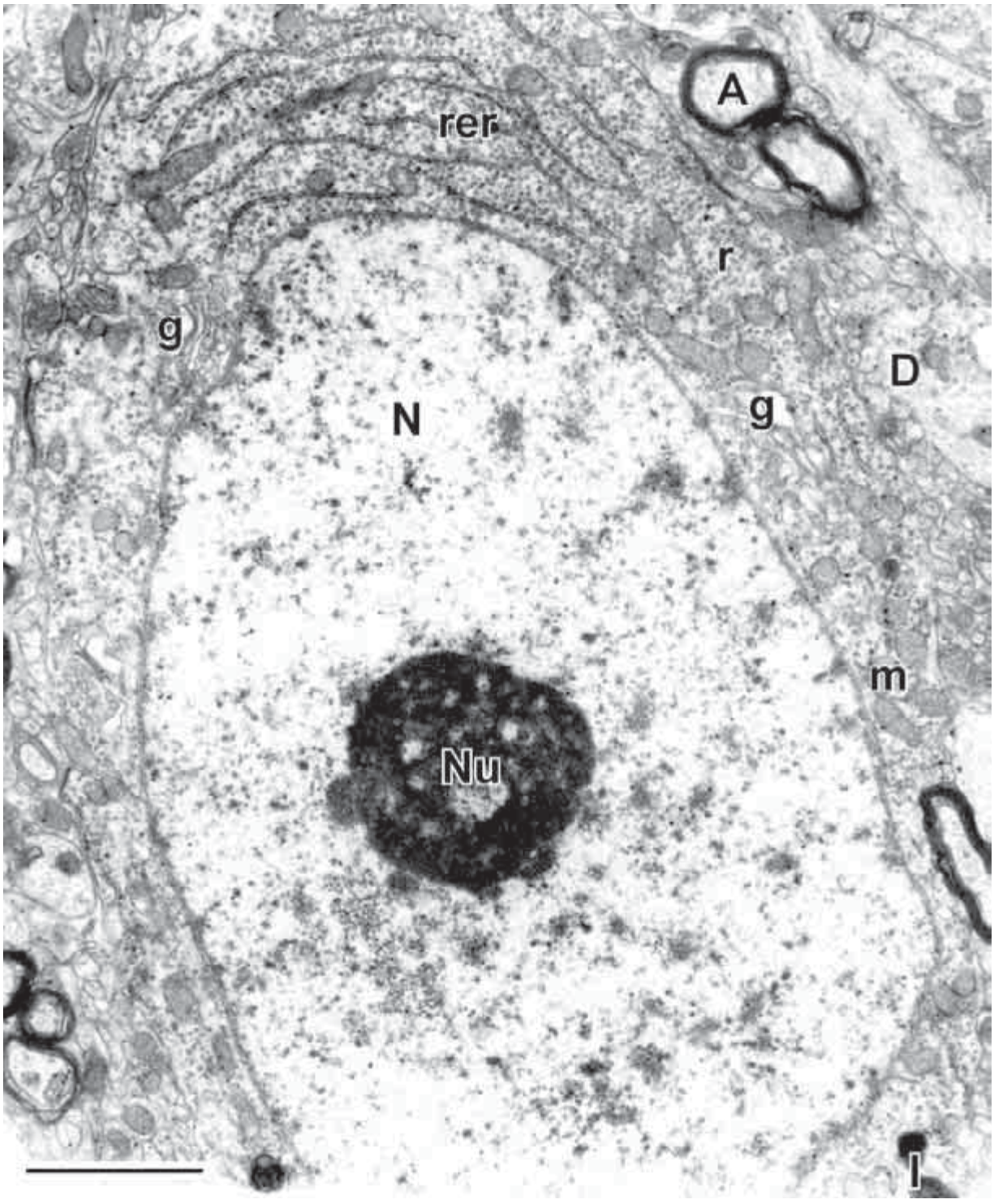

Figure 1. Control (normally fed) young rat. The large nucleus (N) contains prominent nucleolus (Nu) surrounded by slightly invaginated nuclear envelope. The cytoplasm contains a variety of organelles: cisternae of rough endoplasmic reticulum (rer), Golgi complexes (g), ribosomes (r), mitochondria (m) and lysosomes (l). Myelinated axons (A) and dendrites can also be recognised (D). Bar: 2 μm.

ual bodies, typical for age-related neuronal changes [3]. However, VMN neurones of old rats additionally differed from cells of young animals in the close apposition of nucleoli to the nuclear envelope, and in the peripheral localisation and loose arrangement of RER cisternae. Although hypothalamic VMN was long ago proposed to function as "satiety centre" [1], and this hypothesis gained further support [4,

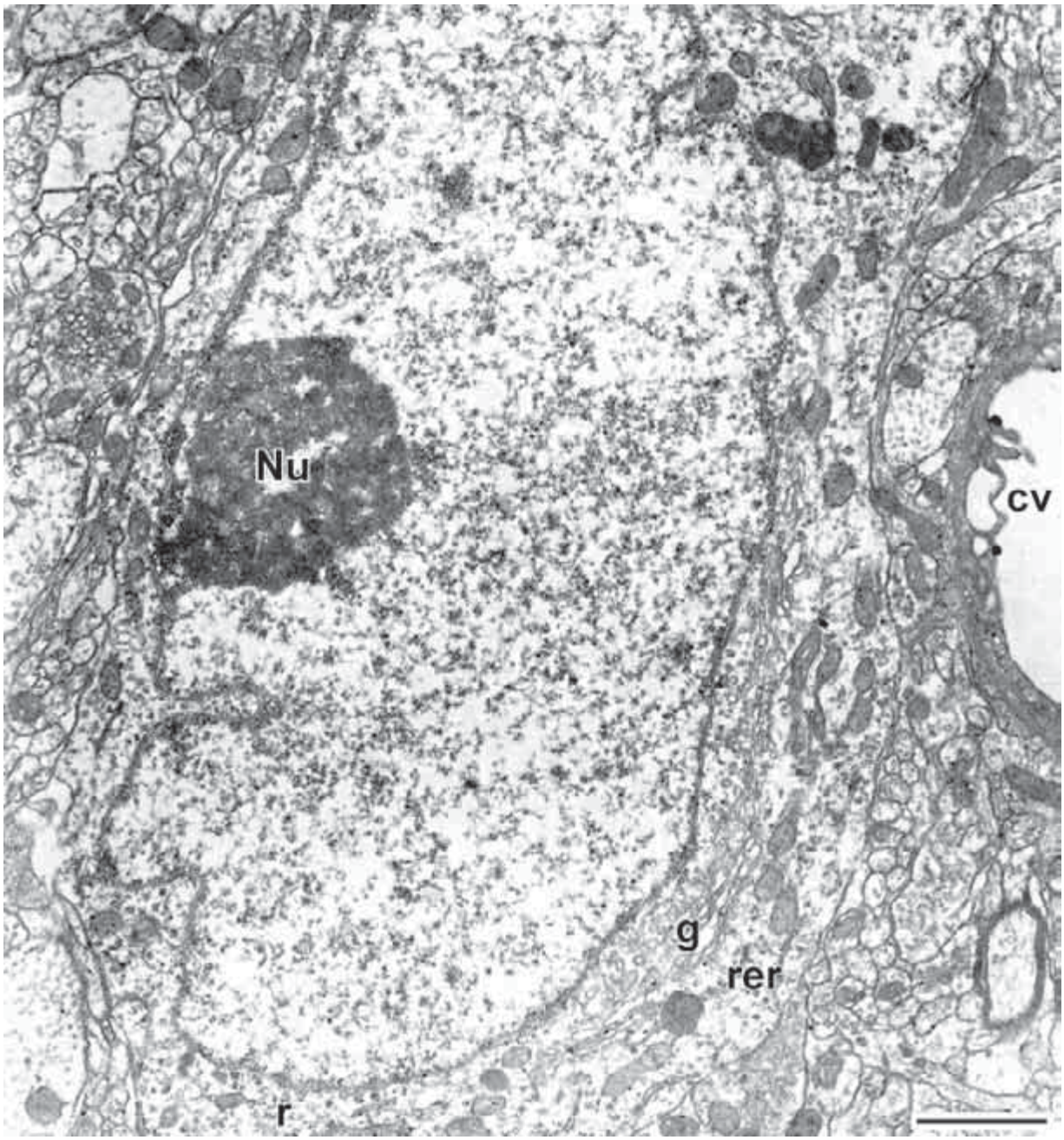

Figure 2. Young rat fasted for 48 h. The prominent nucleolus (Nu) lies close to the nuclear envelope that shows moderate invaginations. Extensive Golgi complexes (g) are arranged into incomplete perinuclear ring. Small number of RER fragments (rer) and groups of ribosomes (r) are also seen. Capillary vessel (cv). Bar: 1 μm.

6, 15], the VMN neurones do not secrete any known peptide or opioid mediator and seem to function as typical neurones [6]. Thus, the observed ultrastructural alterations suggest that the course and/or efficacy of protein synthesis in the VMN neurones of old rats may be different than in young animals.

The effect of fasting on the ultrastructure of VMN neurones of young and old rats has not been previously described. In both young and old rats fasting for 48 hours led to the expansion of Golgi complexes and cytoplasmic vacuoles. These changes may indicate increased cellular activity of VMN neurones. The localisation of the nucleolus close to the nuclear envelope, the appearance of nuclear envelope indentations and the peripheral arrangement of mitochondria in the cytoplasm seem to support this notion. It is interesting to note that fasting-induced increased activity of VMN neurones took place in the situation

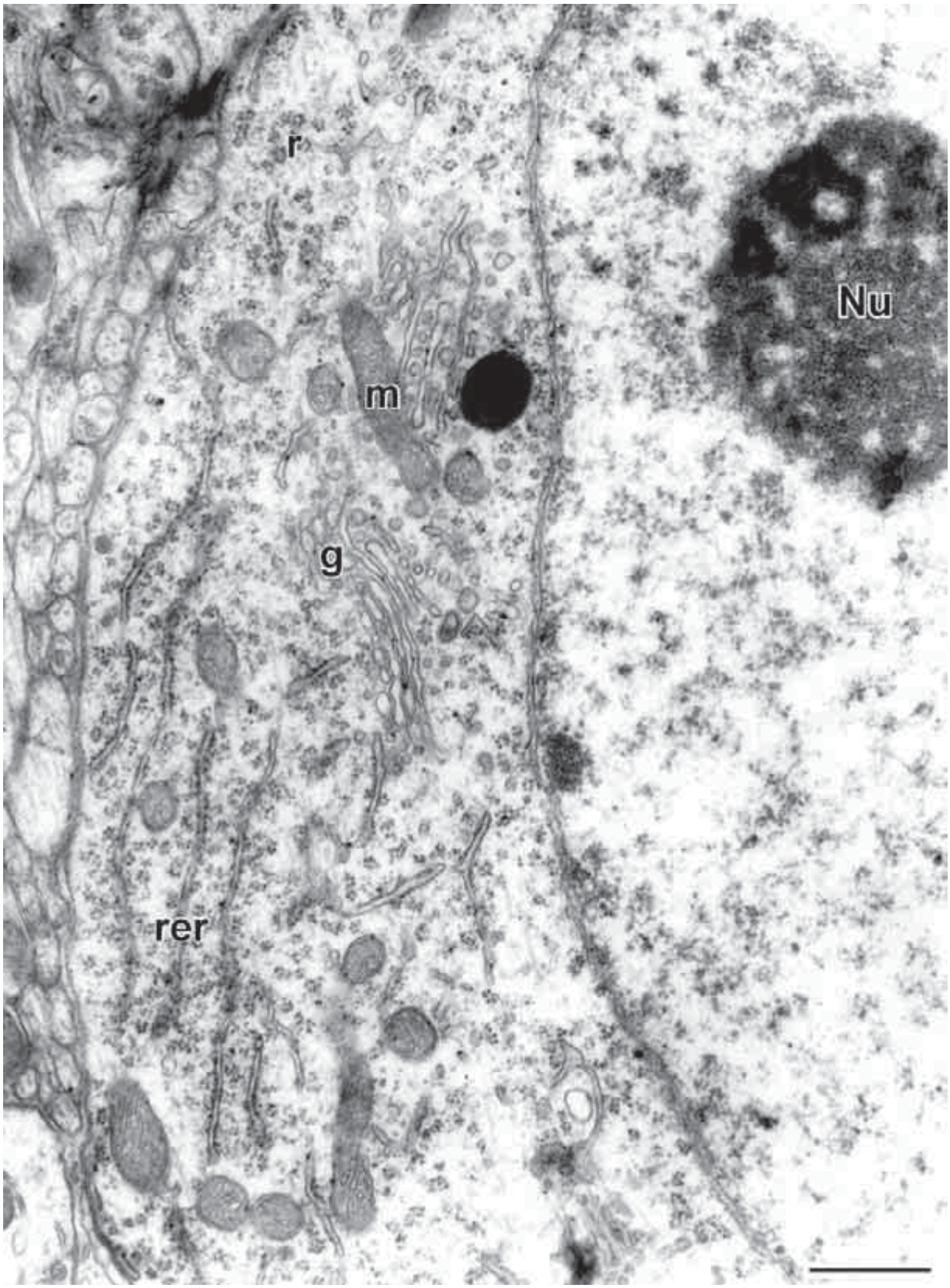

Figure 3. Young rat fasted for 48 h and refed for 24 h. The cytoplasm contains prominent Golgi complexes (g) and numerous polyribosomes (r). Long RER cisternae (rer) and mitochondria (m) are present peripherally. Bar: 500 nm.

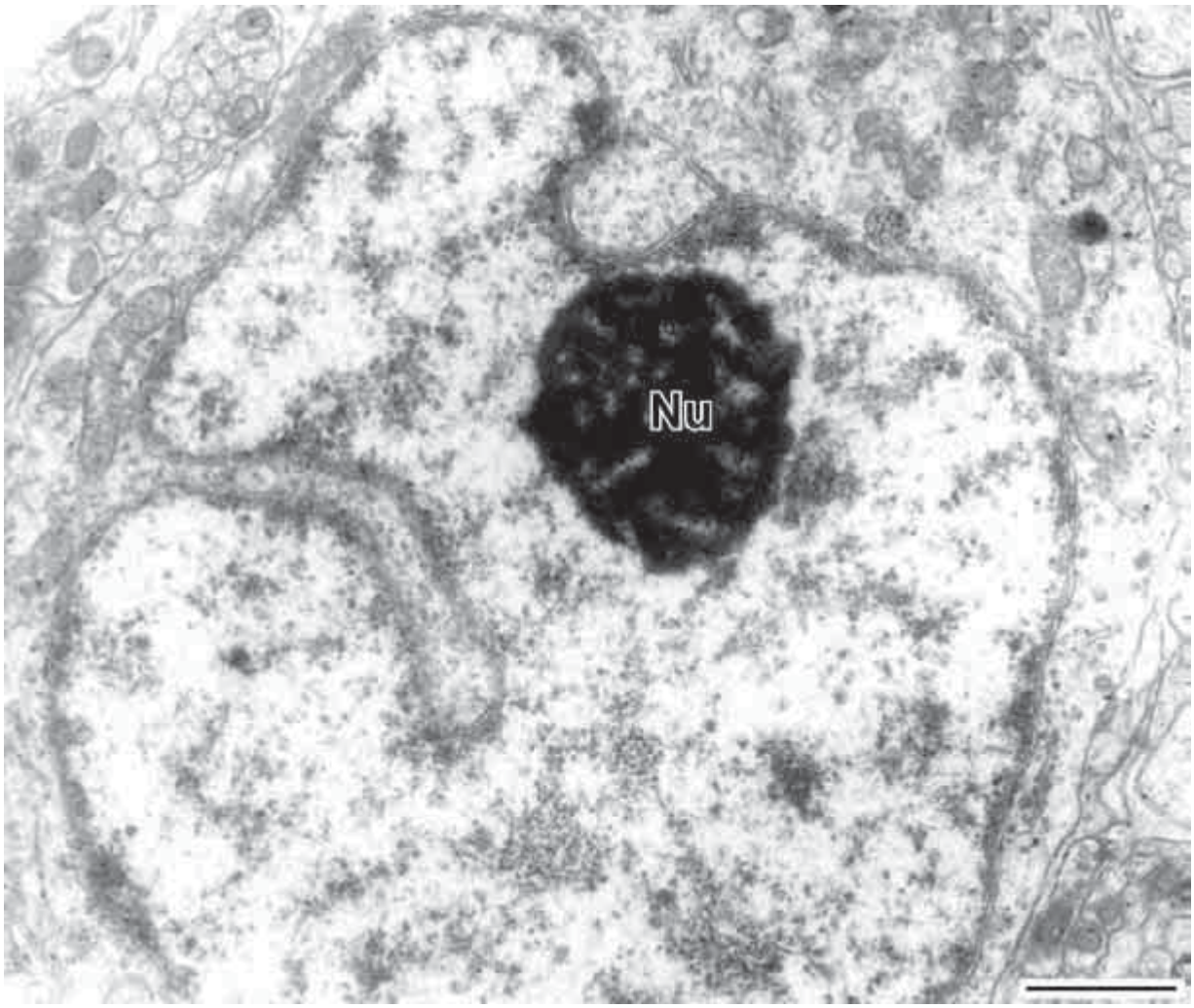

Figure 4. Control (normally fed) old rat. The prominent nucleolus (Nu) is situated close to the invagination of the nuclear envelope. Bar: 1 μm.

when the physiological activity of the VMN neurones, i.e. the restriction of the feeding behaviour, should be suppressed during starvation.

We are not aware of any studies of the ultrastructure of VMN neurones in refed rats. Our observations suggest that in the refed state both in young and old rats the activation of VMN neurones observed in fasted animals still persists. This is evidenced by the prominent expansion of Golgi compexes and RER cisternae in the peripheral cytoplasm. Thus, our ultrastructural investigations suggest that despite age-related differences in the ultrastructure of the VMN neurones of control rats, fasting and refeeding in a similar way increase the cellular activity of the "satiety centre". The observed lower food intake of refed old rats cannot be related to the age-associated morphological alterations of the VMN neurones.

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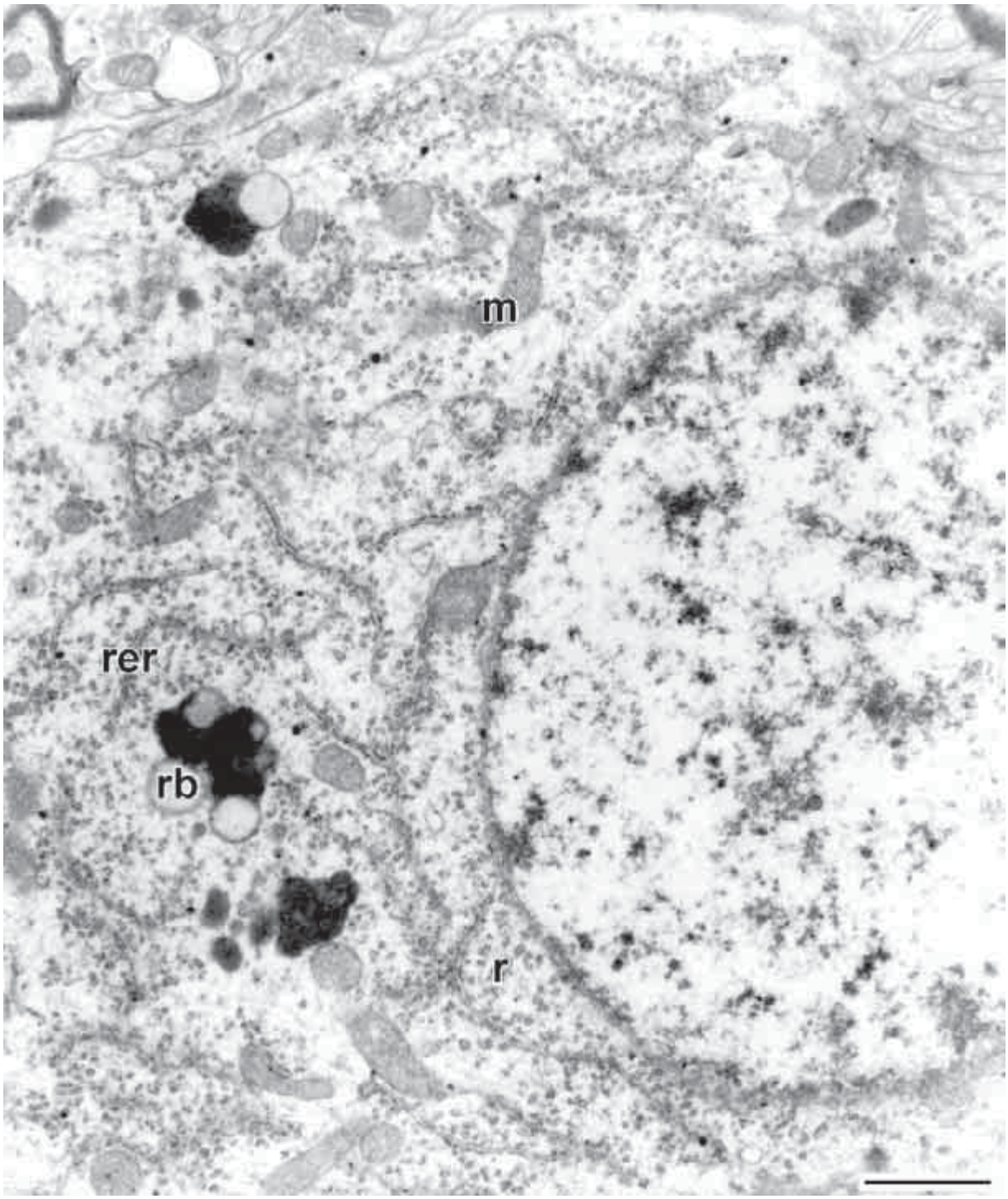

Figure 5. Control (normally fed) old rat. Long undulating RER cisternae (rer) and mitochondria (m) are loosely dispersed in the cytoplasm. Multiple free ribosomes (r) form many rosettes. Residual bodies (rb) are clearly visible. Bar: 1 μm.

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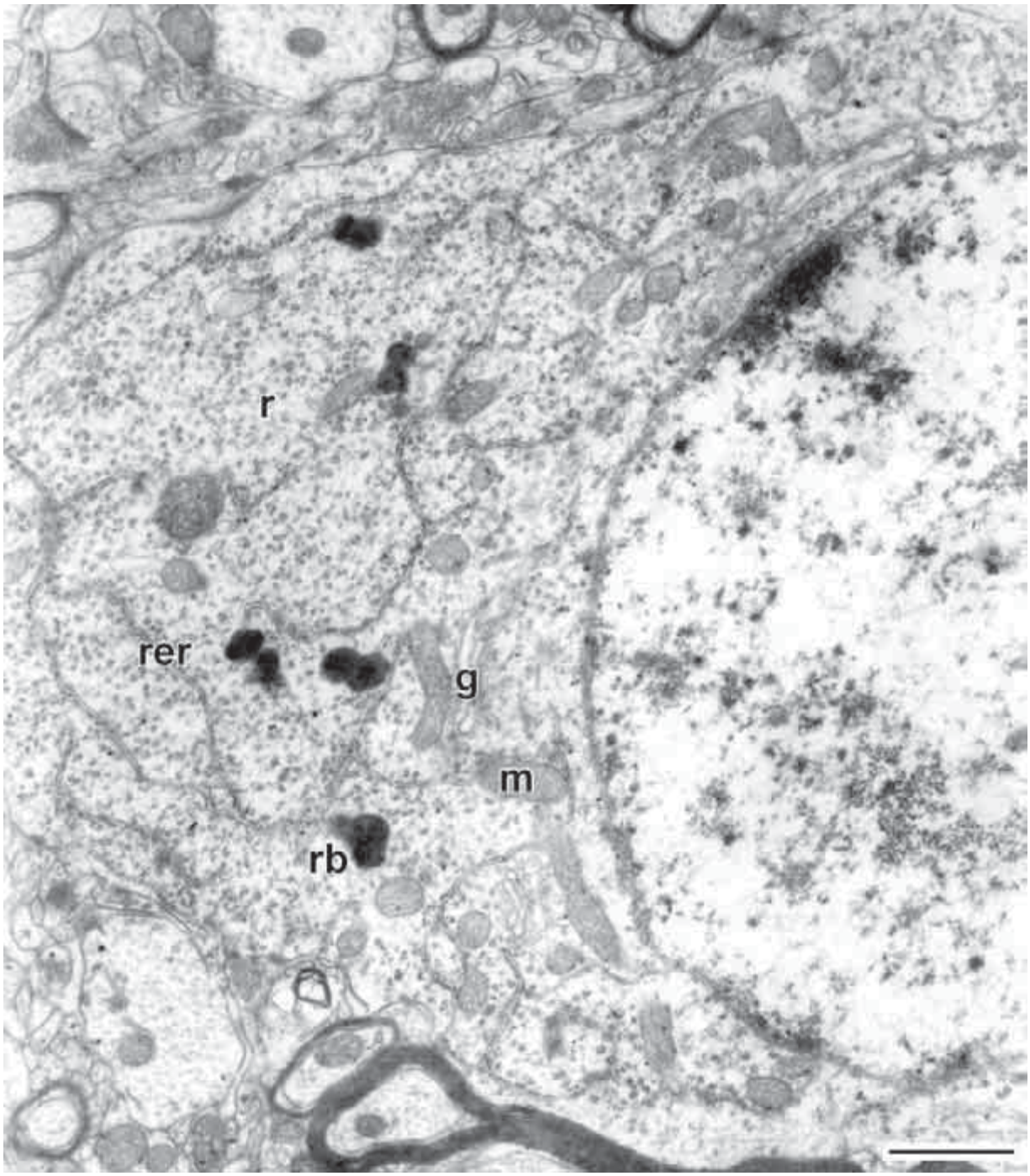

Figure 6. Old rat fasted for 48 h. The cytoplasm contains RER in the form of long, undulating cisternae (rer), abundant polyribosomes (r), extensive Golgi complexes (g), mitochondria (m), and residual bodies (rb). Bar: 1 μm.

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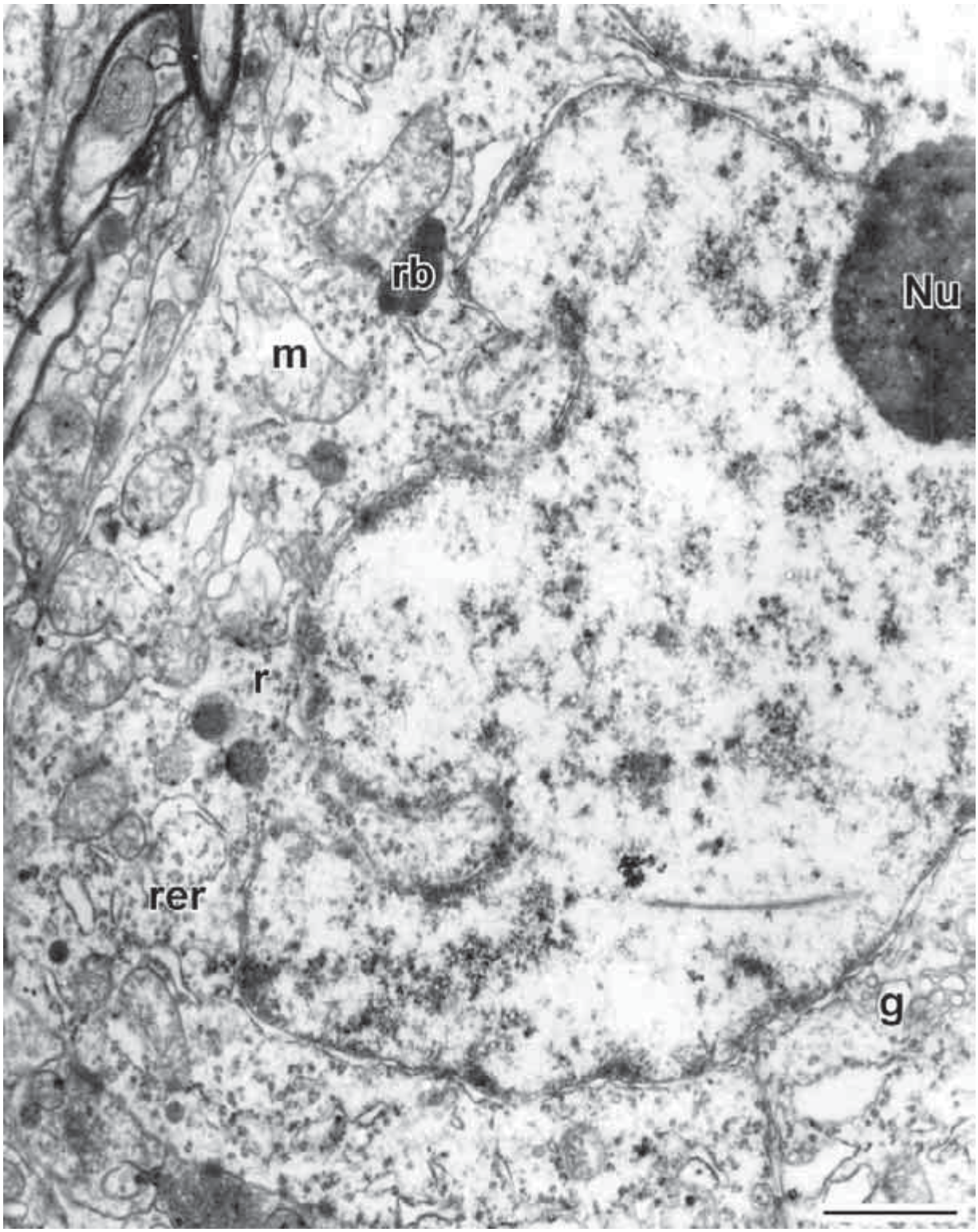

Figure 7. Old rat fasted for 48 h and refed for 24 h. Nucleolus (Nu) is situated near the deep invagination of the nuclear envelope. RER cisternae (rer) are in the form of short dilated fragments. Polyribosomes (r) fill the cytoplasm. Golgi complexes (g) are composed of slightly dilated sacs and vesicles. Swollen mitochondria (m) and pleomorphic residual bodies (rb) are present throughout the cytoplasm. Bar: 1 μm.

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