THE AMERICAN JOURNAL OF ANATOMY 160:363-379 (1981)

Effects of Glucagon on Hepatic Glycogen and Smooth Endoplasmic Reticulum

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ABSTRACT The action of glucagon on hepatic glycogen and smooth endoplasmic reticulum (SER) was studied in samples of liver taken sequentially from anesthetized rats. The physiological state of each animal was assessed by continuously monitoring aortic blood pressure and blood lactate/pyruvate ratios. High hepatic glycogen levels were established by using 10–12 hr fasted control-fed rats infused continuously with glucose. In rats receiving glucose only, hepatic glycogen levels remained above 5.0% during the 4-hr period of glucose administration. Centrilobular hepatocytes displayed an abundance of glycogen which often appeared dispersed with elements of SER between the glycogen particles. Periportal cells had dense clumps of glycogen with few vesicles of SER restricted to the periphery of the glycogen masses. The addition of glucagon to the glucose infusate caused a marked stimulation of glycogenolysis. In these rats, the hepatic glycogen level ($\bar{X} \pm SE$) was $6.71 \pm .15\%$ 1 hr after glucose and declined after initiation of glucagon infusion as follows: $5.86 \pm .29\%$ (15 min), $4.89 \pm .26\%$ (1 hr), $2.16 \pm .40\%$ (2 hr), and $1.66 \pm .29\%$ (3 hr). The fine structure of hepatocytes showed a dramatic response to the administration of glucagon. The glycogen regions of the cells were noticeably decreased in size and number of glycogen granules 3 hr after initiation of glucagon infusion, and SER was abundant in both periportal and centrilobular hepatocytes. The interpretation offered is that glucagon induces the formation of new SER membranes which participate in glycogen breakdown and/or glucose release from hepatocytes.

It has been shown that proliferation of smooth endoplasmic reticulum (SER) occurs in association with the decline in liver glycogen during fasting (Cardell, 1977). In conjunction with these morphological studies, the microsomal location of glucose-6-phosphatase provides biochemical evidence for functioning of the SER in hepatic glucose release. During fasting-induced loss of liver glycogen, basal levels of insulin decrease while portal blood glucagon concentration increases (Unger and Orci, 1976). These changes, i.e., lowering of the portal blood insulin/glucagon ratio, may thus provide the proper hormonal milieu favoring synthesis of SER membranes rich in glucose-6-phosphatase activity, thus facilitating hepatic release of free glucose which maintains the blood sugar level during fasting. Similarly, rats made diabetic with streptozotocin are hyperglucagonemic (Pagliara et al., 1975), and the livers of such animals show abundant quantities of SER which contain increased amounts of microsomal glucose-6-phosphatase (Garfield and Cardell, 1979). Proliferation of smooth endoplasmic-reticulum membranes in response to elevated portal blood glucagon concentration would be consonant with the demonstrated ability of the hormone to stimulate *de novo* synthesis of several enzyme proteins (Shih and Chan, 1979; Wicks, 1974). In this regard, the effects of starvation on malic enzyme synthesis in the liver appear to be mediated by glucagon (Goodridge, 1978). In rats maintained on a controlled feeding cycle, SER (measured morphometrically) is at its lowest level 12 hours after initiation of a 2-hr meal and gradually increases as glycogen loss progresses with continued fasting (Cardell, 1977). Whether these changes can occur on a shorter time frame or are part of the rapid glycogenolytic response to glucagon stimulation *in vivo* is not known. On the contrary, most ultrastruc-

Received July 14, 1980. Accepted December 12, 1980.

0002-9106/81/1604-0363$05.00

tural studies have dealt with the ability of the hormone to induce autophagocytosis with little attention being given to the morphological changes associated with glucagon driven glycogenolysis, a bonafide physiological action of the hormone. Thus, the aim of this investigation was to develop an *in vivo* rat model which would allow a more complete evaluation of the early morphological changes occurring in hepatocytes during glucagon-stimulated glycogenolysis, with particular attention given to the involvement of the smooth endoplasmic reticulum in this process.

MATERIALS AND METHODS

Male Wistar rats weighing between 300 and 350 gm were used in these studies. The animals were housed in individual cages with wire-meshed floors to prevent coprophagy, and water was available at all times. Lighting was regulated to provide 12 hr of light (0700–1900) and 12 hr of darkness (1900–0700), and room temperature was maintained between 21.1–23.9°C. The animals were maintained on a controlled feeding regimen for at least ten days prior to use. Daily food (Purina rat-chow pellets) intake was restricted to the 2-hr period between 2200 and 2400. Under these feeding conditions rats learn to consume large quantities of food during the 2-hr interval when it is available. The livers of such animals contain maximum quantities of glycogen between 10 and 12 hr after initiation of feeding (Cardell, 1977). The duration of these experiments was 5 hr; this included a 2-hr period for surgery and stabilization and a 3-hr experimental period. Surgery was started at 0700 and required 40 to 60 minutes. Liver sampling began 2 hr later or at about 0900 (i.e., 11 hr after initiation of feeding).

Surgical techniques

Under light ether anesthesia, animals were anesthetized by injecting pentobarbital (40 mg/kg body weight) into the left saphenous vein. Laparotomy was performed by means of a midline incision along the linea alba extending from the symphysis pubis to the sternum and two lateral incisions completely exposing the splanchnic viscera. The inferior vena cava was cannulated (PE-50) via the left inferior external pudendal vein using standard procedures. Heparin (50 Units) was injected. The abdominal aorta was cannulated (PE-50) using a method described by Weeks and Jones (1960). A saline-filled needle (23 gauge) was inserted into the iliocolic vein for infusion of glucose. In four rats an additional catheter

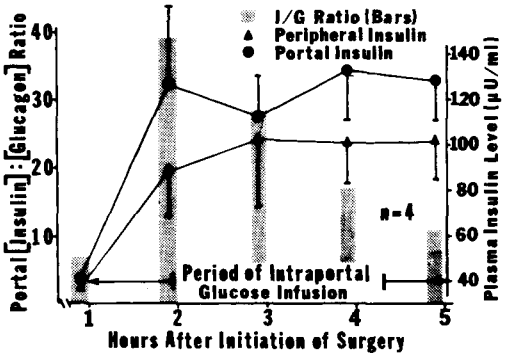

Fig. 1. Insulin-glucagon dynamics in the glucose-infused anesthetized rat. Blood samples were drawn from either the hepatic portal vein (●—●) or the inferior vena cava (▲—▲) at hourly intervals just prior to liver biopsy. Mean values (±SE) of peripheral and portal insulin plasma concentrations are recorded for each time point. The associated portal plasma glucagon levels (pg/ml) were 144 ± 18 (1 hr), 156 ± 46 (2 hr), 164 ± 44 (3 hr), 460 ± 241 (4 hr) and 466 ± 102 (5 hr). Insulin/glucagon ratios are shown as bars (stippled).

(PE-10) was passed into the portal vein for collecting blood for insulin/glucagon ratio determinations (Fig. 1). Upon completion of surgery, the rat was transferred to a prewarmed heating pad; and small amounts of pentobarbital (2.5 mg) and morphine (0.1 mg) were administered periodically thereafter as needed. Aortic blood pressure was recorded continuously with a Statham strain gauge and a Beckman pen recorder (Type RB Dynograph). Occasional rats showing sustained hypotension ($\overline{BP} < 90$ mm Hg) were terminated immediately. In addition to continuous measurement of blood pressure, which served as an acute index of physiological state, the aerobic state of each animal was also assessed by continuous monitoring of the blood lactate/pyruvate ratio. Ratios between 10 and 20 were taken to indicate the existence of normal respiratory function. Data obtained from rats showing a persistent elevation of the lactate/pyruvate ratio were discarded.

During each experiment, five liver samples were sequentially removed[1]. Liver lobes (or portions of lobes) were always removed in the same order as follows: The portion of the cystic lobe to the left of the ligamentum teres hepatis was taken as the baseline sample (pre-gluca-

1. In these studies, it was assumed that the liver lobes are homogenous with respect to glycogen level. Thus, the glycogen concentration measured in any individual lobe is representative of the entire liver. The validity of this assumption has experimental justification in data provided by others (Corrin et al., 1968). In addition, manipulation of the liver during lobe removal appeared to have no effect on the glycogen level of lobes remaining in the animal. Indeed, in livers exposed to lower concentrations of glucagon (41 ng/min), the 15-min hepatic-glycogen level showed no change from the baseline (pre-glucagon) value (data not shown).

gon in the hormone-infused rats); thereafter, the posterior portion of the right lobe, the anterior portion of the left lobe, and finally the remainder of the cystic lobe were removed. After ligation and removal, a small portion of each liver sample was prepared for electron microscopy as described below. The remainder was frozen between two blocks of dry ice and stored frozen (−80°C) for glycogen assay.

Glucose and glucagon infusion

In all animals postprandial glucose levels were maintained by continuous intraportal infusion of a 1.39 M glucose solution (Krebs-Ringer bicarbonate containing 1% bovine serum albumin), starting about one hr after beginning surgery and continuing until termination of the experiment. The rate of infusion was 13.8 mg/min for 5 min and 9.3 mg/min thereafter. The protocol was identical in the glucagon-infused rats except that glucose solution containing glucagon (2.8 μg/ml) was infused commencing at two hr. The rate of hormone delivery used (137 ng/min) appeared to be about the minimum required to produce an immediate decline in the hepatic glycogen level (15-min post-glucagon sample) in spite of the existing hyperinsulinemia (Fig. 1). The plasma glucagon concentration produced in the portal blood during infusion of the hormone was approximately 20 ng/ml.[2] All solutions were infused with a Harvard pump (Model H975).

Tissue Sampling Protocol and Assays

In the hormone-stimulated rats, a baseline liver sample was removed after one hr of glucose infusion (zero hour). Glucagon administration was started immediately; and liver samples were obtained at 15 min, 1 hr, 2 hr, and 3 hr (Fig. 2). In animals infused with glucose only, samples were obtained at 0, 1, 2, and 3 hr. Liver glycogen levels (% hepatic glycogen) were measured in duplicate using methods described previously (Cardell, 1977).

Peripheral blood insulin concentrations and portal blood insulin/glucagon ratios were measured in samples of blood drawn just prior to initiation of glucose infusion and every hour thereafter immediately preceding liver sampling. Plasma insulin concentration was determined using a charcoal separation procedure (Albano et al., 1972). Plasma glucagon was measured with 30K antibody in accordance with the method of Faloona and Unger (1974). Blood glucose and lactate/pyruvate ratios were measured in samples of blood drawn

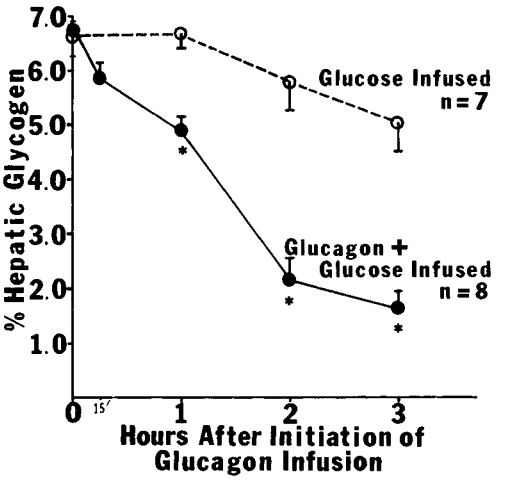

Fig. 2. Effect of intraportal glucagon infusion on hepatic glycogen levels. All rats were continuously infused with glucose (9.3 mg/min) commencing 1 hr prior to removal of the baseline liver sample at zero hour. Hepatic glycogen level is expressed as the mean % of wet liver weight (±SE) for each time point. In the hormone-infused animals (glucagon + glucose), the average rate of glycogen degradation during the 2-hr period of glucagon responsiveness was 0.36 (mg/min)/gm liver ($r = 0.978$, regression line not shown). (*): $p < .001$ as compared to levels measured in rats infused with glucose only.

from the vena cava at 30 min and 1 hr; and, upon initiation of intraportal glucose infusion, at 15-min intervals. Blood glucose was determined using the glucose oxidase method of Krebs et al. (1964). Lactate/pyruvate ratios were calculated from metabolite concentrations measured enzymatically (Bücher et al., 1965; Hohorst, 1965). Student's t-tests were used in all statistical comparisons with p values of $< .05$ taken to be significant.

Electron and Light Microscopy

(See Cardell, 1977 for details). Immediately after removal, a small portion of the liver sample was placed in 2% glutaraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer at pH 7.3 (with 2-4 mM of calcium chloride added). The tissue was further subdivided into small cubes of 1 mm^3 and smaller. The minced tissue was kept in fixative at room temperature for 2 hr, washed in buffer, postfixed in 1%

2. The actual portal plasma concentration measured in five blood samples collected from one animal over the 3-hr experimental period was $X \pm SE = 21.9 \pm 2.2$ ng/ml.

3. See footnote 1.

OsO_4 for 2 hr, dehydrated in ethanol, and embedded in Epon mixture, or held in the primary fixative at 4°C for later processing (within two weeks). Semithin sections of each specimen were cut, placed on glass slides and stained with 0.5% toluidine blue in 1% borax solution for identification of central veins and portal tracts. Blocks were then trimmed for ultrathin sectioning to include at least one portal tract and/or one central vein. The sections were stained with uranyl acetate and lead citrate. Both centrilobular and periportal areas were studied ultrastructurally in all samples.

RESULTS

Just prior to initiation of glucose infusion, the mean peripheral blood glucose concentration was 144 mg/dl. A 5-min pulse of glucose (13.8 mg/min) followed by constant infusion of the sugar (9.3 mg/min) raised the peripheral level by about 75 mg/dl. Samples of blood taken at 30-min intervals thereafter had a mean value (±SE) of 219±20 mg/dl in rats infused with glucose only (n=7). The associated portal plasma glucose concentration was in the postprandial range (298±61 mg/dl, n=5). In addition, continual intraportal glucose infusion and the associated hyperglycemia resulted in the establishment of a high portal plasma insulin/glucagon ratio and a peripheral hyperinsulinemia (Fig. 1). Portal plasma glucagon concentration remained at about 150 pg/ml until the third hour but increased three-fold thereafter, which lowered the insulin/glucagon ratio (Fig. 1).

Maintenance of a high portal plasma glucose concentration and elevation of the insulin/glucagon ratio favored glycogen deposition and stasis of liver glycogen. Thus, as shown in Figure 2, in rats infused with glucose alone, the mean glycogen level (±SE) was 6.62±0.38% at zero hour. No change was seen 1 hr later, but the level gradually declined thereafter reaching 5.05±0.55% by the end of the fourth hour of glucose infusion. In the hormone-infused animals, the zero-hour mean hepatic-glycogen level was 6.71±0.15%. After only 15 min of glucagon infusion, the percent liver glycogen declined significantly to 5.86±0.29% ($p < .02$) as compared to the value at zero hour.[3] In these rats, the hepatic glycogen level declined steadily during the first 2 hr of glucagon infusion. The average rate of glucose release during this period was 0.40 (mg/min)/gm wet liver (determined by dividing the rate of glycogen degradation by 0.9). As shown in Figure 2, between hours 2 and 3, the decrease in hepatic glycogen in the glucagon-infused rats was actually less than that observed in rats infused with glucose only, signifying hepatic resistance to glucagon. In conjunction with declining liver glycogen levels, glucagon caused a sustained elevation of the blood glucose (255±18 mg/dl, n=7) above that recorded in animals infused with glucose only, although these differences were not statistically significant.

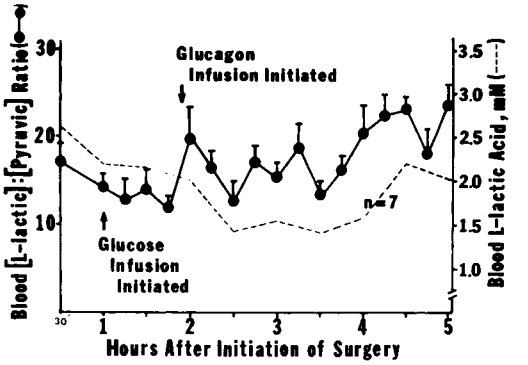

Fig. 3. Blood L-lactate pyruvate levels in anesthetized rats infused with glucose and glucagon. Blood lactate/pyruvate ratios are recorded as mean values (±SE) (●—●). Changes in the mean L-lactic acid concentrations in the 30-min blood samples are also shown (----------).

Blood lactate/pyruvate ratios and L-lactic acid levels in rats infused with glucose and glucagon are shown in Figure 3. The occurrence of lactacidemia observed in rats infused with glucose only (data not shown) was prevented by infusion of glucagon with the mean L-lactate concentration being on the average 50% lower in the hormone-infused animals ($p < .01$). Pyruvic-acid levels were similarly affected and ranged from 0.9 to 0.13 mM during glucagon infusion. As shown in Figure 3, mean blood lactate/pyruvate ratios tended to remain between 10 and 20 during the entire procedure indicating the maintenance of normal aerobic state.

As noted previously, the hepatic glycogen level in samples of liver taken after 1 hr of glucose infusion (or approximately 13 hr after initiation of feeding) has a mean value of 6.62% (Fig. 2). This value is comparable to that reported previously for the livers of control-fed rats 10 to 12 hr after initiation of feeding when hepatic glycogen is maximum (Cardell, 1977). In agreement with the chemical measurements, examination of electron micrographs of

hepatocytes from samples taken at this time showed cells with large quantities of glycogen composed of typical α and β particles (Figs. 4,5,6). The appearance of these cells was normal in all respects, and few lysosomes were seen. Centrilobular cells (Figs. 4,5) were characterized by small mitochondria and abundant tubular SER. The SER was distributed primarily in regions of dispersed glycogen (Fig. 5); however, even in regions of densely packed glycogen, elements of the SER were present between the glycogen particles. In contrast, periportal cells contained larger and more rounded mitochondria and tightly packed masses of glycogen (Fig. 6). Much less SER was found in periportal cells, and elements of this organelle were restricted generally to the periphery of the glycogen masses.

Intraportal infusion of glucagon results in an immediate decline in the level of hepatic glycogen (Fig. 2). Comparison of electron micrographs (Fig. 7) of this group with those from animals infused with glucose only indicate that glycogen loss was especially pronounced in centrilobular hepatocytes. Thus, after 15-min exposure to glucagon, centrilobular cells were characterized by an increase in the number of regions which contained highly dispersed glycogen granules. Between the granules of glycogen numerous tubules and vesicles of SER were found (Fig. 7). It was obvious that membranes of the SER were closely associated with glycogen granules in the regions of dispersed glycogen; however, not all the glycogen was dispersed at this time period (Fig. 7). Since the number and size of dispersed regions of glycogen increase in the centrilobular cells after glucagon stimulation and since these regions contain abundant SER, it is clear that SER increases in these cells after glucagon stimulation.

Although the mean hepatic glycogen level decreased by nearly 13% (Fig. 2), this change was not readily apparent in periportal hepatocytes (Fig. 8). Large masses of densely packed glycogen continued to occupy restricted areas of the cytosomes of these cells. In a few periportal cells, dispersed glycogen particles associated with elements of the SER were seen at the periphery of the large glycogen masses although some cells showed regions of dispersed glycogen within the cytosome (Fig. 8). In these regions SER was abundant and showed a close relationship to glycogen particles.

After one hour of glucagon stimulation, most of the centrilobular and midlobular hepatocytes showed a marked response to the hormone. The glycogen regions were characterized by dispersed glycogen particles with only occasional dense clumps (Fig. 9). Within the dispersed glycogen regions, abundant elements of SER were found. The net effect of increased numbers of centrilobular and midlobular cells responding to glucagon and more areas within the responding cells showing dispersed glycogen and increased SER undoubtedly accounts for most of the glycogenolysis occurring during this period of hormone action. As seen in Figure 10, cells in the periportal region remained relatively unresponsive to glucagon stimulation during the time interval studied here. Large regions of the cytosome continued to show masses of densely packed glycogen, although more glycogen areas within the cells showed dispersed glycogen particles and associated SER as described earlier.

Three hr after glucagon infusion almost all cells have lost considerable glycogen, and both centrilobular and midlobular hepatocytes showed extensive accumulations of SER (Fig. 11). Periportal cells contained small clumps of glycogen with SER restricted to the edges of the glycogen masses (Fig. 12). It is clear that periportal cells contain much less SER than centrilobular hepatocytes (compare Figs. 11 and 12). Numerous lysosomes were seen in both centrilobular and periportal hepatocytes after glucagon stimulation.

DISCUSSION

In order to study the action of glucagon on hepatic glycogenolysis, it was important to use rats with high initial hepatic glycogen levels. This was achieved by utilizing animals on a controlled feeding regimen (Cardell, 1977). Postprandial portal glucose levels were maintained by continuous glucose infusion which tended to stabilize hepatic glycogen levels in control animals. In addition, the high systemic blood glucose levels stimulated insulin release and suppressed glucagon secretion, shifting the insulin/glucagon ratio in favor of glycogen deposition (Cherrington et al., 1979; Parrilla et al., 1974; Unger and Orci, 1976) and maintenance of high hepatic glycogen levels. The underlying enzymatic events responsible for these glucose and hormone effects have been well documented (Stalmans, 1976). In the experiments described in this report, continuous intraportal glucagon infusion caused a sustained lowering of the insulin/glucagon ratio, which favored high rates of glycogenolysis and

progressive loss of liver glycogen. Thus, we were able to study this process at specific intervals with chemical and morphological techniques.

It is generally held that glucagon has no effect on carbohydrate metabolism in the peripheral tissues (Dunn and Chenoweth, 1979). Thus, the pronounced lowering of the blood L-lactate and pyruvate concentrations observed during infusion of glucagon were assumed to be due to stimulation of hepatic gluconeogenesis by the hormone (Cherrington et al., 1979; Unger and Orci, 1976; Williamson, 1966). Maintenance of the blood concentrations of these metabolites near resting levels in spite of their apparently high rates of production by the peripheral tissues additionally suggests that efficient operation of glucagon-stimulated gluconeogenesis occurred in the animals used in this study. The apparent preservation of this sensitive liver function indicated that the general state of the livers of the animals used in this study was good. In addition, the existence of normal aerobic metabolism is underscored by the observation that venous blood lactate/pyruvate ratios remained in the normal range during the entire procedure. The lactate/pyruvate ratio in rat blood is typically near 20 (Marbach and Weil, 1967) with values between 10 and 20 taken to be physiological (Ross et al., 1967). Electron microscopy was performed only on samples of liver tissue taken from rats meeting this criterion. It is also noteworthy that a marked lowering of the blood lactate and pyruvate levels was observed in all glucagon-infused animals studied.

Under maximal glucagon stimulation, the perfused rat liver releases glucose at average rates between 0.70 and 0.80 (mg/min)/gm wet liver (Sokal and Weintraube, 1966; Striffler and Curry, 1979). Peak values of 1.0 (mg/min)/gm, near the maximum possible for the liver's enzymatic machinery, are measurable. In the glucagon-infused animals, the average rate of glucose release was 0.40 (mg/min)/gm wet liver during the period of glucagon responsiveness. Thus, infusion of the hormone at 137 ng/min produced portal plasma glucagon levels near 20 ng/ml (5.7×10^{-9}M), which resulted in approximately half maximal rates of glycogen degradation. As suggested from studies with *in vitro* and *in vivo* systems (Cherrington et al., 1979; George and Bailey, 1978; Unger and Orci, 1976), the portal plasma glucagon concentration of 20 ng/ml used in these experiments was about ten-fold higher than physiological levels. However, in view of the existing hyperinsulinemia incident to glucose infusion, the portal insulin/glucagon ratio, which is generally taken to be the actual determinant of hepatocyte response (Parrilla et al., 1974; Unger and Orci, 1976), was well within the physiological range. This point is reinforced by the observation that rates of glycogen degradation in response to glucagon were about half-maximal (Parrilla et al., 1974; Sokal and Weintraube, 1966; Striffler and Curry, 1979). Thus, the changes in glycogen and SER observed in this study are not pharmacological responses but represent physiological mechanisms involved in glucagon-stimulated hepatic glycogenolysis.

In general, the observations conform with well-documented views about the glycogen-mobilizing action of glucagon in the liver (Cherrington et al., 1979; Parrilla et al., 1974; Sokal and Weintraube, 1966; Striffler and Curry, 1979; Unger and Orci, 1976). Thus, sustained elevation of the portal glucagon concentration (low insulin/glucagon ratio) resulted in continuous hepatic release of glucose. The absence of any inhibitory effects of hyperglycemia evident in this study is in agreement with *in vivo* observations made by others (Gilboe and Nuttall, 1978), although some investigators have reported that glucose has inhibitory effects on glucagon-stimulated glycogenolysis (see discussion in Reference 1). The existence of hepatic resistance to glycogen depletion in control-fed animals has been reported by a number of investigators (Babcock, 1974). Accordingly, the complete absence of responsiveness of the liver to glucagon during the third hour may be part of this metabolic adaptation to controlled feeding. The most significant point emerging from these comparisons is, however, that glucagon responses of the livers of these animals are similar to those recorded with other *in vivo* systems.

In order to interpret correctly liver morphology, particularly at the ultrastructural level, careful control of several variables is

Abbreviations

GA	Golgi apparatus	N	nucleus
GL	glycogen (densely packed)	RER	rough endoplasmic reticulum
LY	lysosomes	SER	smooth endoplasmic reticulum
M	mitochondria		

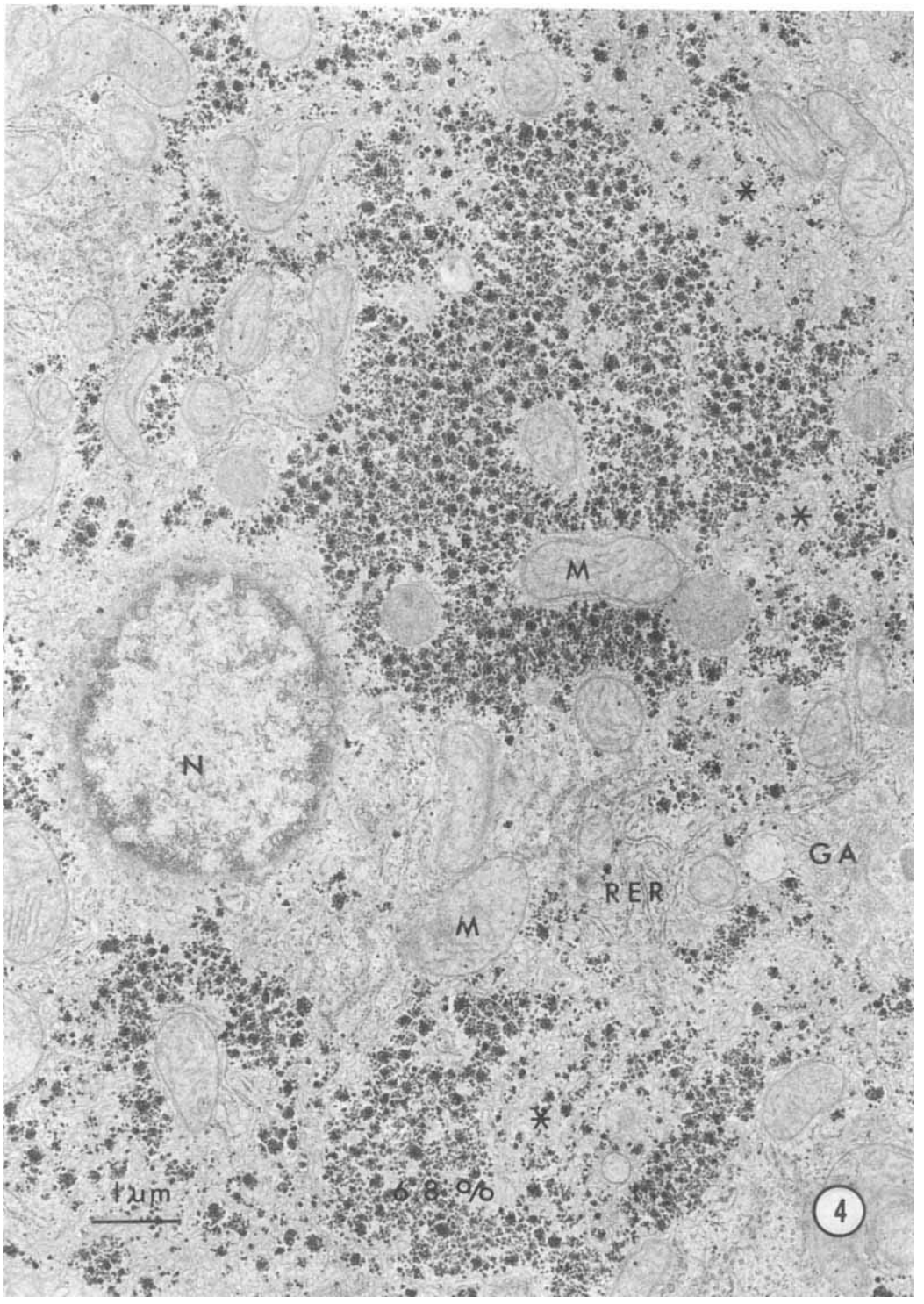

Fig. 4. Electron micrograph of a centrilobular hepatocyte from the liver of an anesthetized rat infused intraportally with glucose for 1 hr. This sample, the first of a sequential sampling from the same liver, was removed at zero hour just prior to the initiation of glucagon infusion (refer to Figure 2). The chemically measured hepatic glycogen concentration was 6.8%, and dense patches of glycogen in this centrilobular cell reflect the relatively high level of hepatic glycogen. Other areas of the cytosome (*) contain glycogen granules scattered among tubules of smooth endoplasmic reticulum. × 13,600.

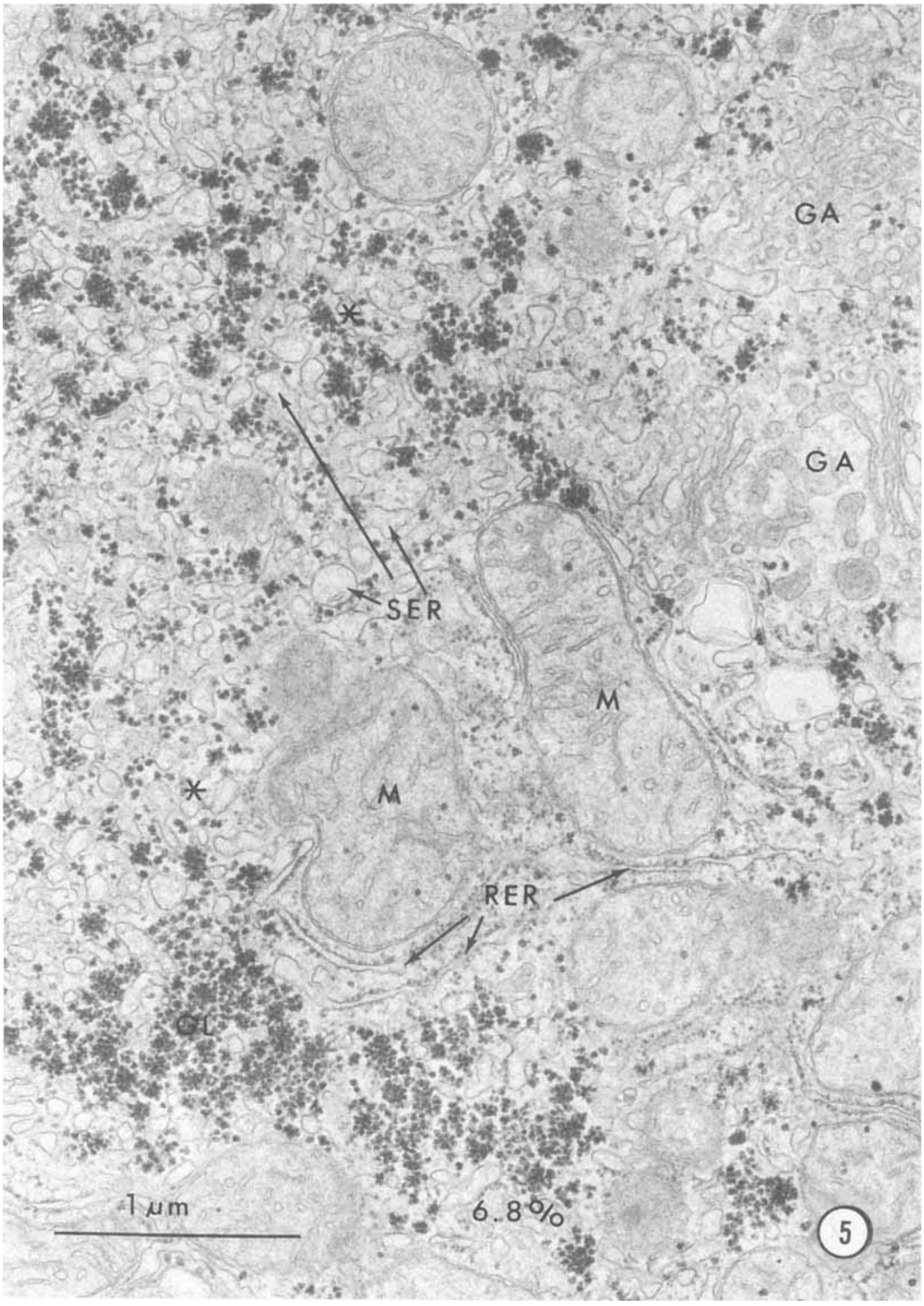

Fig. 5. This electron micrograph is a higher magnification of a centrilobular cell from the same sample as in Figure 4. A region of the cytosome which contains primarily dispersed glycogen (*) among elements of SER is shown. × 37,400.

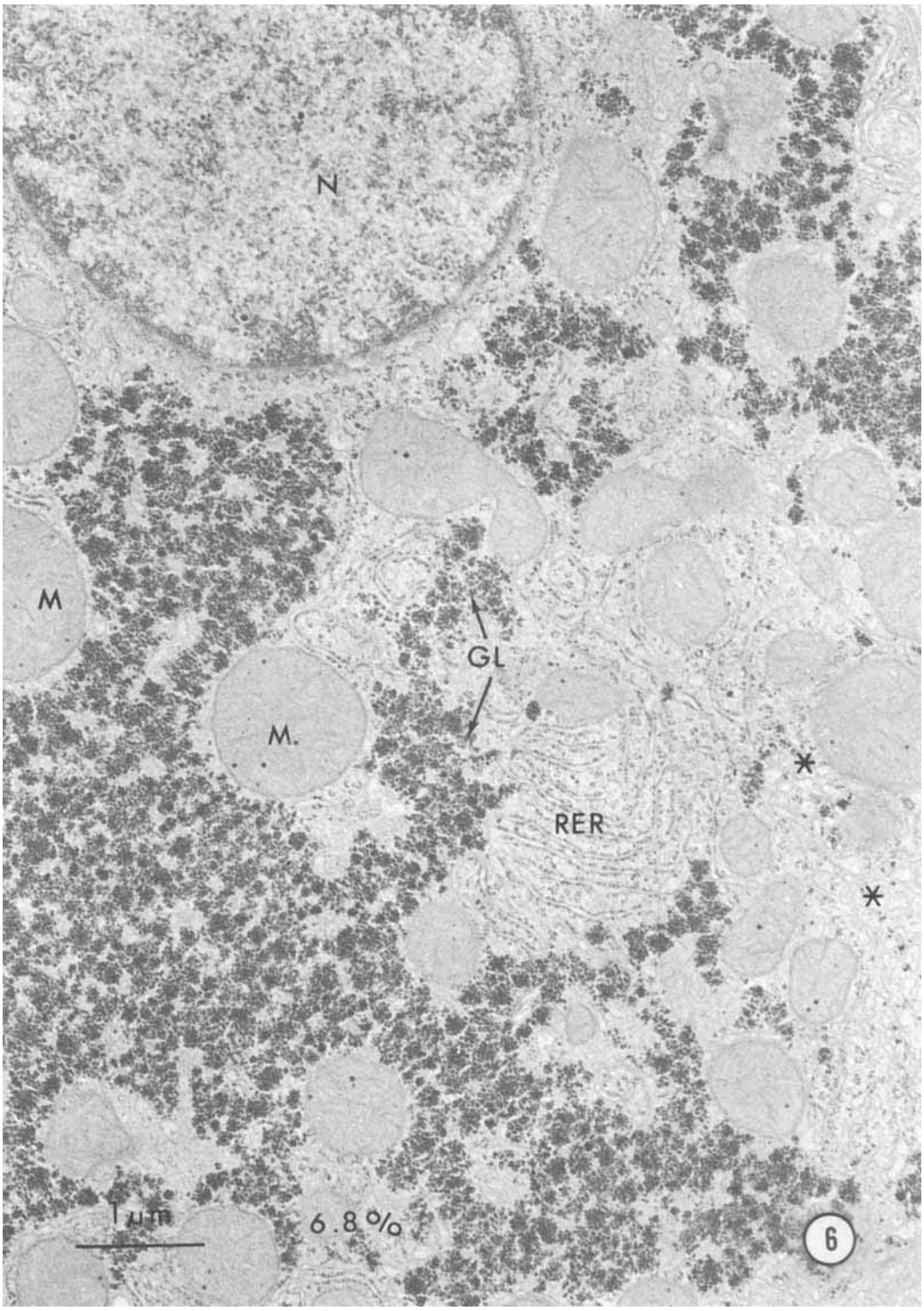

Fig. 6. A periportal hepatocyte from the same sample as in Figures 4 and 5 (1 hr of intraportal glucose infusion, just prior to glucagon infusion; chemically measured hepatic glycogen level 6.8%). This micrograph illustrates the large dense glycogen mass typical of periportal cells. Relatively small areas of the cytosome contain elements of SER and dispersed glycogen granules (*). Note the lack of SER within the dense clumps of glycogen and the presence of this organelle in regions of dispersed glycogen. × 19,400.

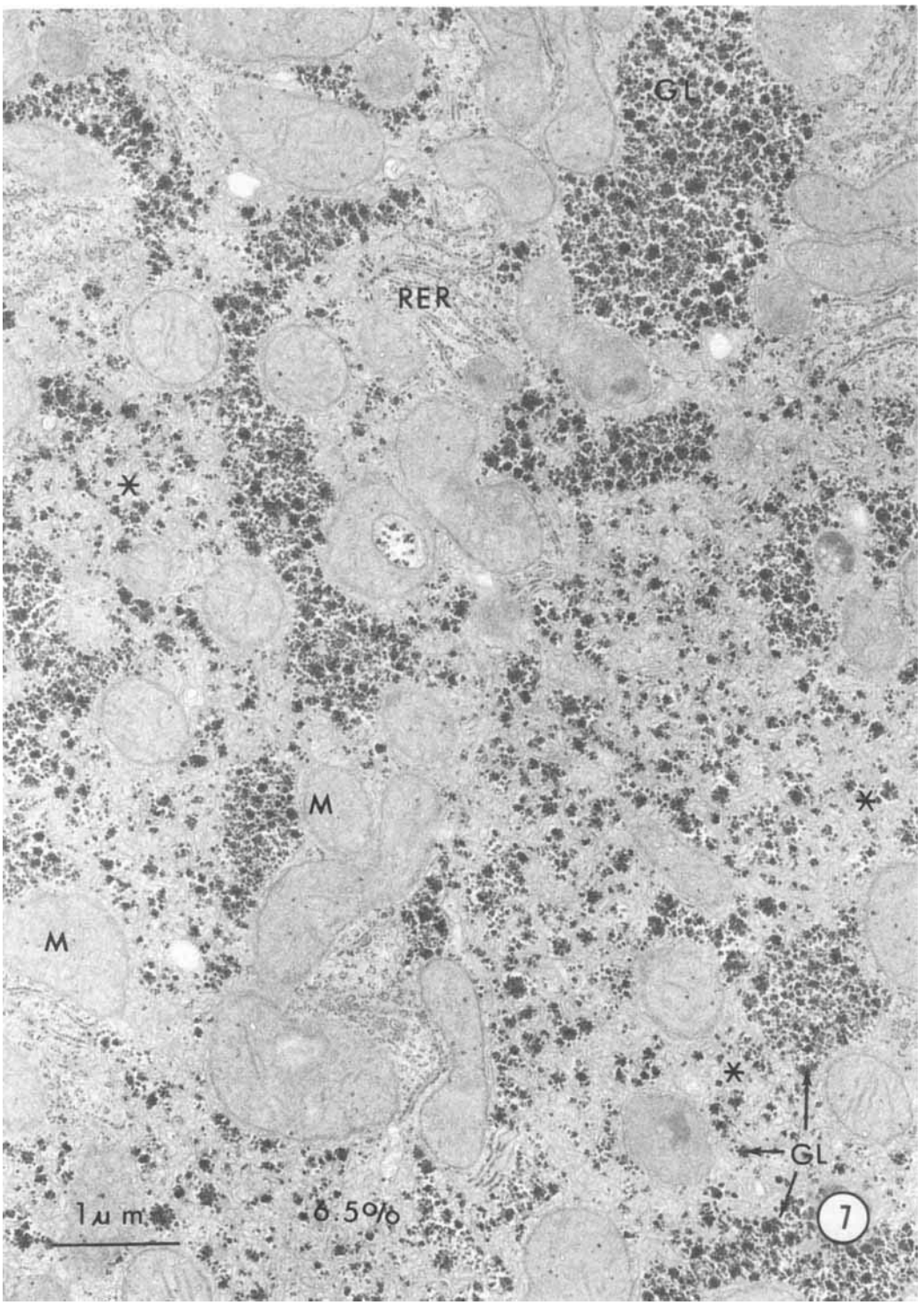

Fig. 7. This electron micrograph was taken of the second sequential sampling (15 min after initiation of glucagon infusion) from the same liver used in Figures 4–6. The chemically measured hepatic-glycogen level decreased from 6.8% to 6.5%. Numerous regions of this centrilobular cell show areas of dispersed glycogen (*) which contain abundant elements of SER. Other regions of the cell show masses of glycogen with small amounts of SER at the periphery of the glycogen mass. × 19,400.

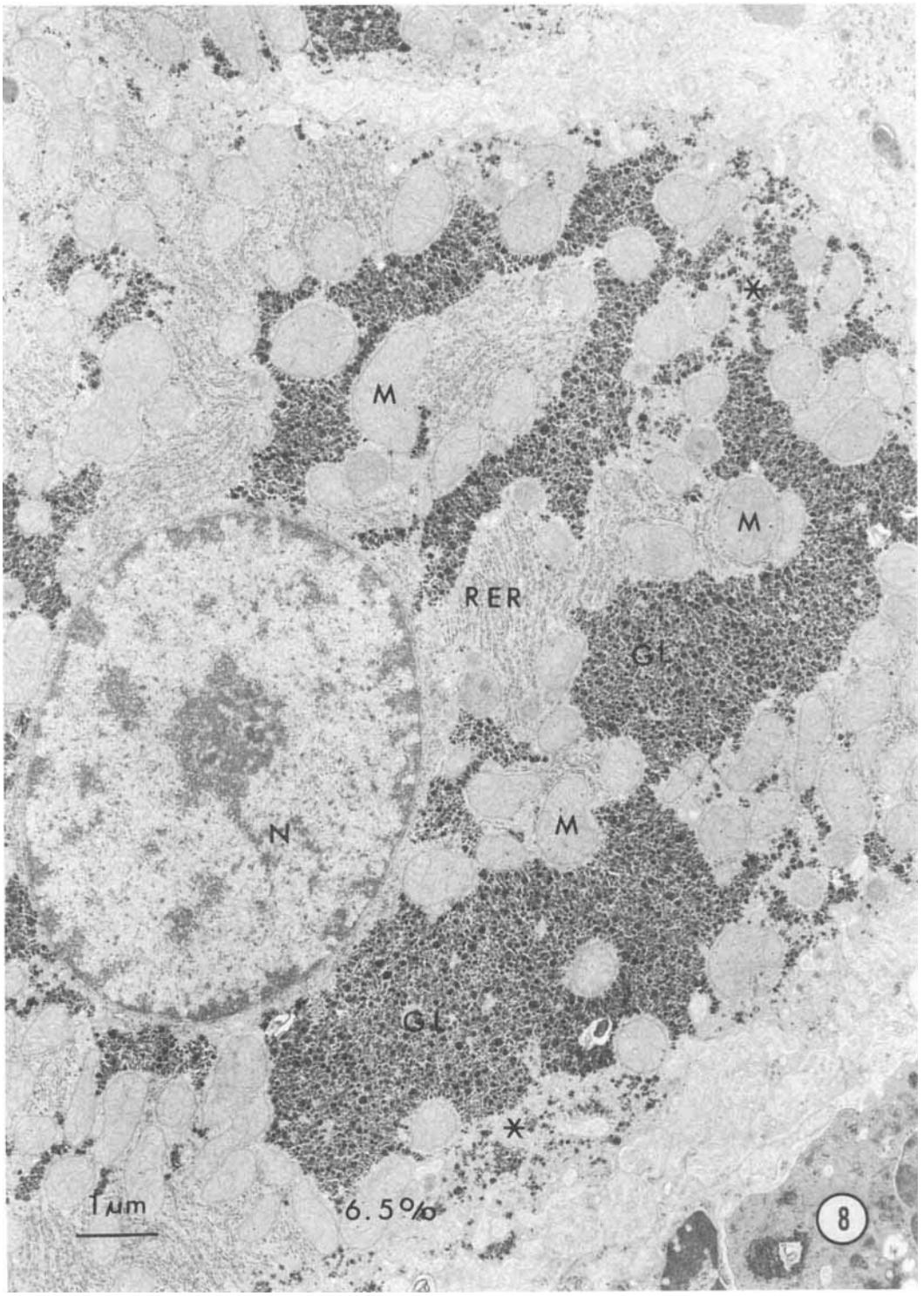

Fig. 8. A periportal hepatocyte from the second sequential sample (15 min of glucagon infusion; chemically measured hepatic glycogen level 6.5%), showing large dense patches of glycogen typical of these cells. A few areas (*), primarily near the cell periphery, contain dispersed glycogen and elements of SER. × 12,200.

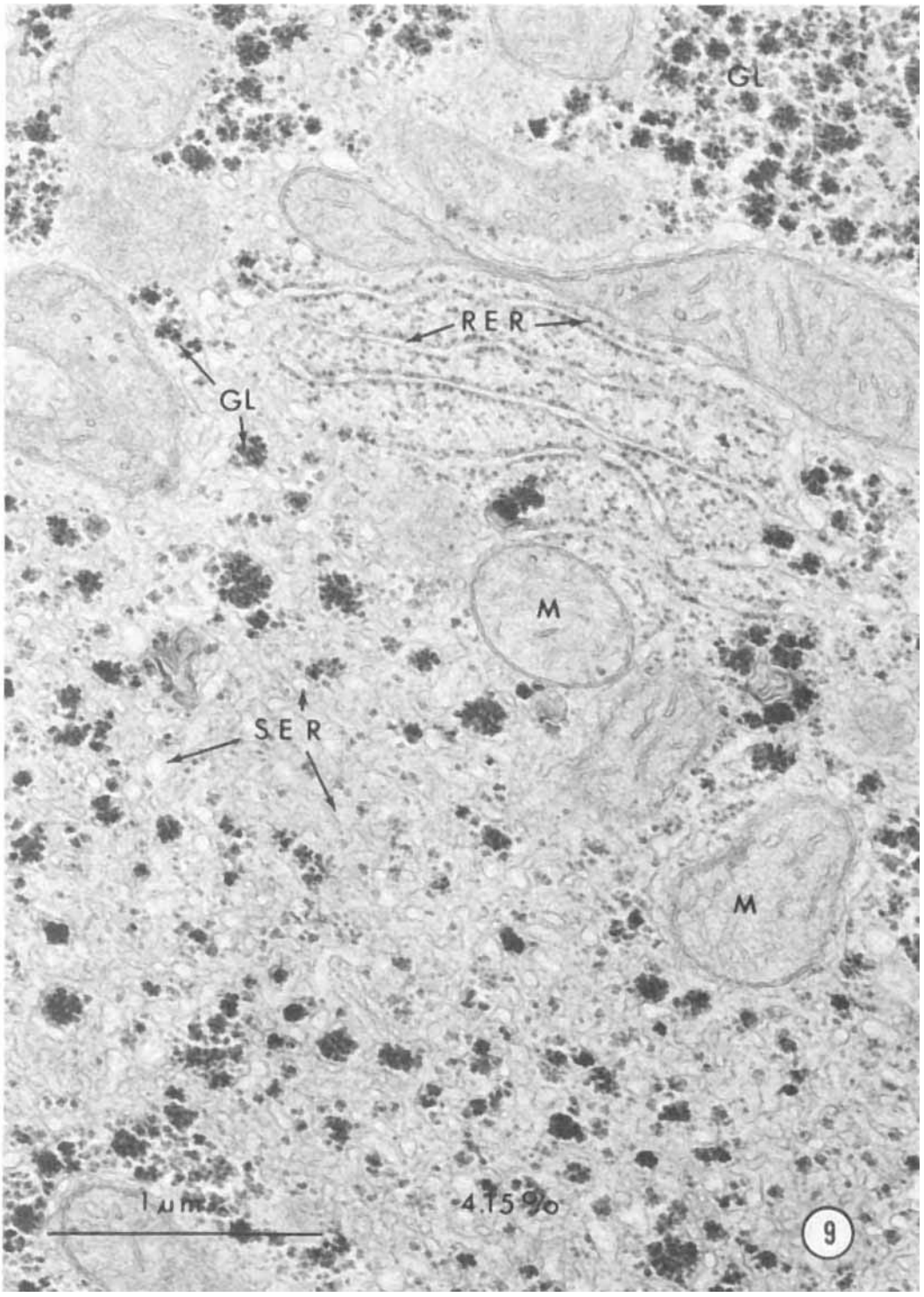

Fig. 9. This electron micrograph is representative of centrilobular cells in a liver sample obtained in the third sampling sequence after 1 hr of glucagon infusion. The sample was obtained from the same liver as Figures 5–8; the chemically measured hepatic-glycogen level at this time was 4.15%. Some regions of the cell have dense glycogen masses with SER at the periphery of the glycogen mass, while the more common distribution for SER is between the dispersed glycogen particles. × 42,000.

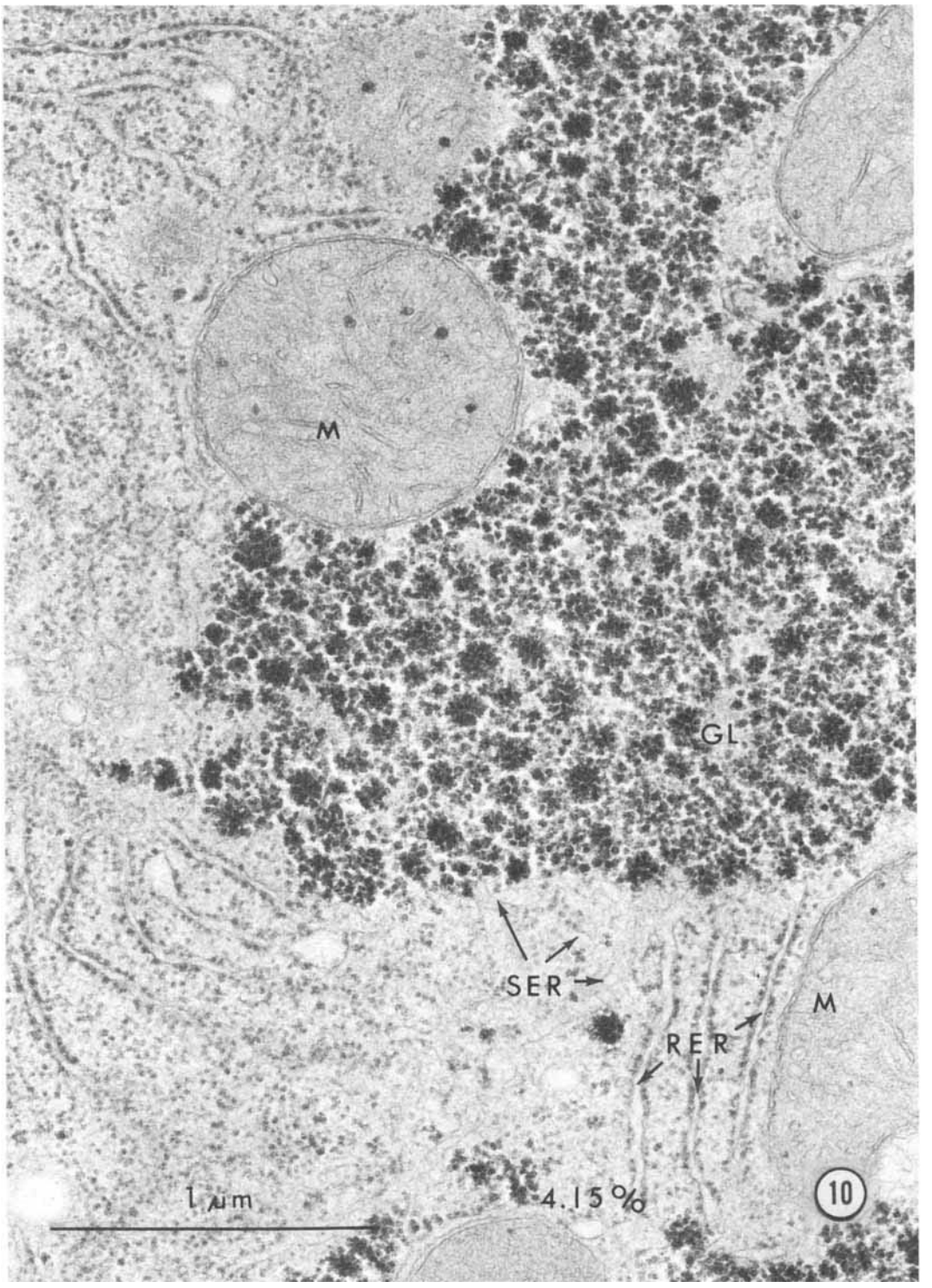

Fig. 10. A periportal hepatocyte after 1 hr of exposure to glucagon (same sequential sample as illustrated in Figure 9). Dense masses of glycogen with peripheral SER are the more common pattern of glycogen; however, some areas of the cytosome show dispersed glycogen among SER tubules. × 49,000.

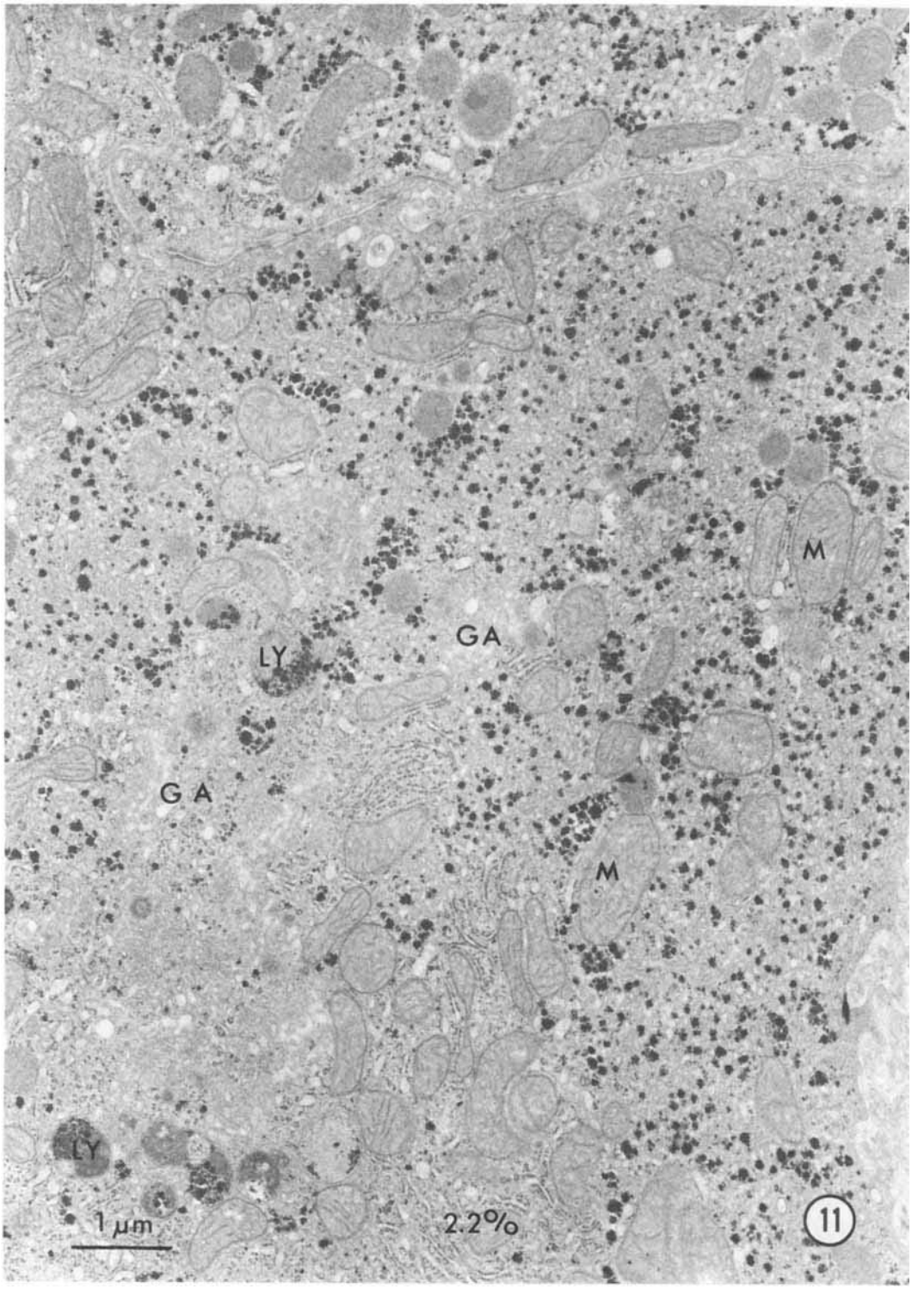

Fig. 11. A centrilobular hepatocyte from the liver of a rat infused intraportally with glucagon for 3 hr. Note the presence of dispersed glycogen infiltrated with SER throughout the cytosome. The chemically measured hepatic glycogen level was 2.2%. × 15,600.

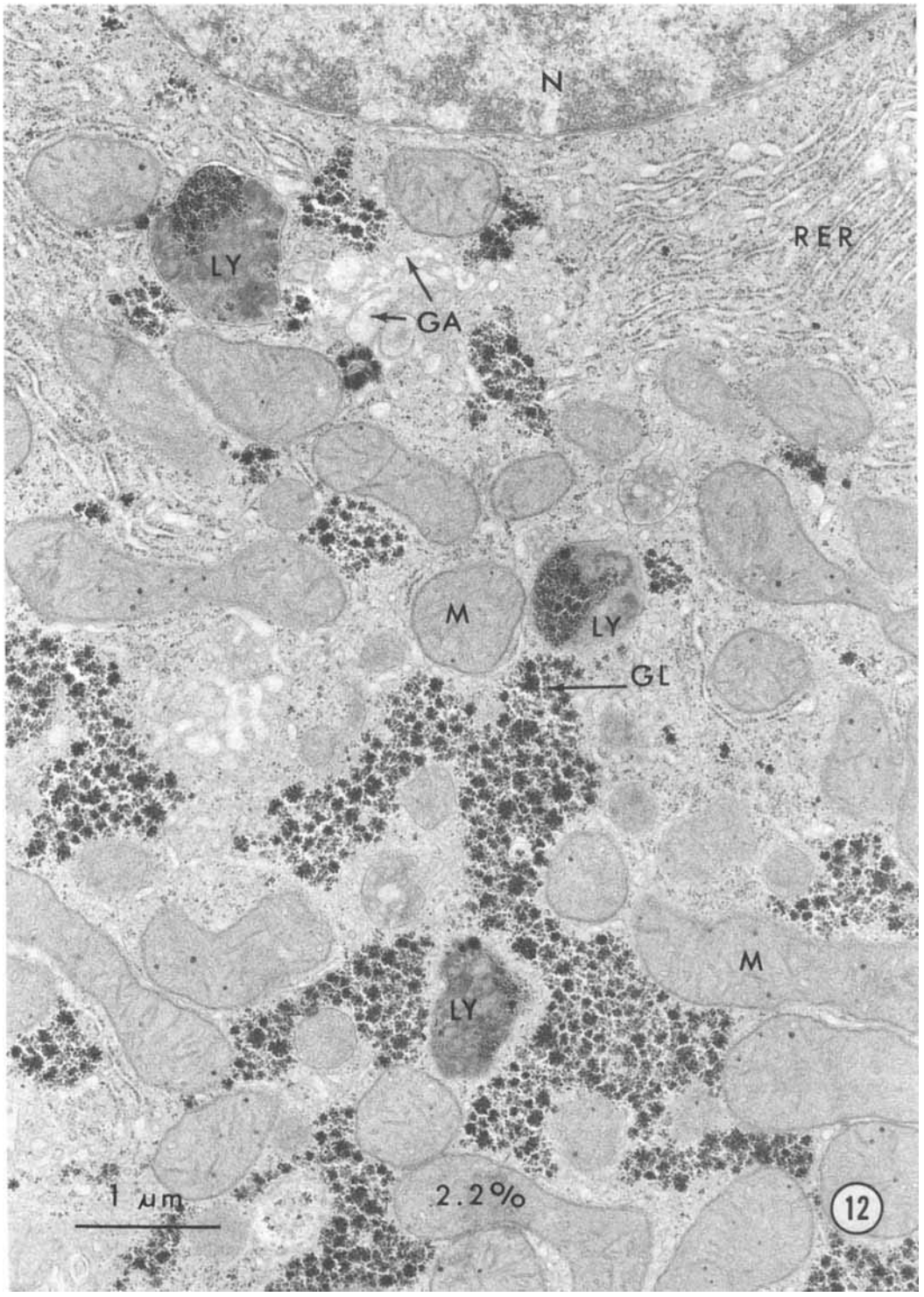

Fig. 12. A periportal cell showing persistent masses of glycogen from a rat liver infused intraportally with glucagon for 3 hr. These masses are smaller than those observed in specimens from earlier samples in the sequence. The chemically measured hepatic glycogen level was 2.2%. Lysosomes are numerous and frequently contain glycogen particles. × 22,000.

necessary. Recognition of this fact is especially important when examining morphological relationships between SER and hepatic glycogen (Cardell, 1977). During the 22-hr fast following a 2-hr meal, the livers of control-fed rats accumulate and then deplete glycogen in a characteristic manner (Cardell, 1977). Precise knowledge of the fasting time of such animals and chemical measurements of hepatic glycogen for each liver studied eliminates much of the variation observed when using *ad lib* fed animals and reliance upon mean glycogen levels. Equally important in studies examining liver-cell morphology is precise knowledge of the location of hepatocytes within the liver lobule. Thus, in the experiments described herein, these variables were minimized by using animals trained on a controlled feeding cycle. Experiments were commenced between 10 and 12 hr after initiation of feeding when hepatic glycogen levels are maximum and SER is at its lowest level (Cardell, 1977). In addition, chemical glycogen measurements were done on each liver sample studied, and the lobular position of hepatocytes selected for electron microscopy was identified as either centrilobular or periportal.

Twelve hr after initiation of a 2-hr meal, the livers of control-fed rats contain maximum quantities of glycogen. As shown previously, lobular patterns of the polysaccharide are clearly defined at specific stages of glycogen deposition and depletion (Cardell, 1977). Thus, periportal hepatocytes from baseline samples of tissue taken after 1 hr of glucose infusion and just prior to initiation of glucagon infusion showed large masses of densely packed glycogen in restricted regions of the cytosome. Small amounts of SER were present with the organelle being located primarily at the periphery of the masses. In contrast, hepatocytes from the centrilobular region were characterized by glycogen regions which contained glycogen in rather dense clumps, as well as regions of dispersed glycogen with abundant SER between the glycogen granules. High levels of glycogen and these characteristic patterns were maintained in livers of rats infused with glucose only (controls) for up to 5 hr.

After only 15 min of glucagon stimulation, centrilobular hepatocytes showed numerous glycogen regions which were infiltrated with SER. In contrast, morphological changes in the periportal cells were minimal at this time point, and no large patches of glycogen infiltrated with SER were seen in these cells. After 1 hr of glucagon infusion, centrilobular cells contained almost exclusively dispersed glycogen regions with abundant SER. In contrast, hepatocytes in the periportal region continued to retain dense masses of glycogen which were, however, reduced in size. Periportal cells generally appeared to show less response to glucagon than centrilobular and midlobular cells, although more glycogen areas within the cells showed dispersed glycogen particles and associated elements of SER as the glucagon infusion progressed.

Differences in the distribution of the SER before glucagon stimulation and the differing glycogen depletion patterns resulting after exposure to glucagon in the cells of the two lobular regions clearly indicate that the efficiency of glucagon-stimulated glucose release is greatly dependent upon the presence of SER. Studies on the mechanism of action of glucagon have, for the most part, concentrated on receptor involvement, c-AMP activation, and the cascade of soluble enzymes which mediate the degradation of glycogen to glucose-6-phosphate (Stalmans, 1976). Little attention has been given to glucose-6-phosphatase except that it is required for liberation of free glucose. The observation that glucagon causes few changes in the pattern of glycogen distribution in the periportal cells (where SER is sparse and located at the periphery of the glycogen masses), and conspicuous changes in centrilobular cells (which contain much SER closely associated with glycogen particles), clearly suggests a major role for the SER in the immediate glycogenolytic response of the liver to glucagon. It is conceivable that the SER acts as one of the limiting components in glucagon-stimulated glucose release. If this were not the case and the rate of glucose release were limited solely by the activity of the soluble enzymes, i.e., the phosphorylase system, one would expect to see early development of dispersed patterns of glycogen in the periportal cells.

Proliferation of the SER during fasting is well documented (Cardell, 1977). The presence of glucose-6-phosphatase on this organelle has clearly established a role for the SER in the release of glucose generated by gluconeogenesis, the activity of which is also elevated during fasting (Unger and Orci, 1976). In the present investigation, the involvement of the SER in glucagon-stimulated glycogenolysis by the livers of fed rats has been additionally demonstrated. Whether the morphological changes recorded after only 15 min represent quantitative increases in the amount of SER remains

to be demonstrated. However, the visual impressions suggest that after 1 hr of hormone stimulation, a marked proliferation and quantitative increase in the amount of SER has occurred. Previous studies have reported that stimulation with pharmacological concentrations of glucagon are associated with hypertrophy of the SER in perfused livers of fed rats 40 min after addition of the hormone (Rosa, 1971).

Many agents, notably the glucocorticoids, have been shown to induce proliferation of the SER (Cardell, 1977). Certain peptides act as inducers in endocrine glands (Cardell, 1977). Glucagon has been shown to stimulate *de novo* synthesis of liver enzymes involved in hepatic carbohydrate metabolism (Shih and Chan, 1979; Wicks, 1974). The findings reported here suggest that elevated portal concentrations of the hormone (or a low insulin/glucagon ratio) may also act as an inducer for smooth endoplasmic-reticulum proliferation in the liver.

ACKNOWLEDGMENTS

This investigation was supported by grants AM-06013, AM-27097 and in part by the University of Virginia Diabetes Research Training Center (AM-22125) from USPHS. The authors express their gratitude to Dr. George E. Vandenhoff of the NIH Diabetes Core Laboratory, University of Virginia, for preparing the insulin and glucagon determinations and to Mrs. Paula Halteman for typing this manuscript.

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