

The Effect of Experimental Diabetes Mellitus and Insulin Replacement on Hepatic Ultrastructure and Protein Synthesis

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ABSTRACT The following study was conducted in order to define the specific alterations in hepatic ultrastructure responsible for the decrease in hepatic protein synthesis associated with experimental diabetes. Rats received intravenous alloxan (70 mg/kg) and 48 h later were either sacrificed or given insulin for 1, 2, 4, 6, or 24 h. Specimens for electron microscopic evaluation and morphometric analysis were taken from the same livers used to isolate ribosomes for measurement of *in vitro* protein synthesis. Our results show that hepatocytes from animals with untreated alloxan diabetes show varying degrees of disorganization and loss of rough endoplasmic reticulum (RER) which is directly related to the severity of the alloxan diabetes. A significant correlation existed between the severity of ultrastructural changes as judged by the loss of both membrane and polysome components of the RER and degree of inhibition of protein synthesis ($P < 0.001$). Abnormalities of hepatic ultrastructure and protein synthesis were reversed within 24 h of insulin administration. The data are consistent with the view that it is the relative decrease in hepatic polysomes that results from the loss of RER in alloxan diabetes that is responsible for the decrease in hepatic protein synthesis.

INTRODUCTION

Previous studies have demonstrated that *in vitro* hepatic protein synthesis was markedly depressed when experimental diabetes mellitus was produced in rats (1, 2). This effect was found to be independent of the method of producing diabetes (2), and it was, therefore, assumed that insulin deficiency was responsible for the observed decrease in protein synthesis. Insulin deficiency has also been shown to affect the characteristics of isolated hepatic ribosomes when they are sedimented through a

continuous sucrose gradient (3, 4), as well as to modify their degree of binding to radiolabeled polyuridylic acid (3). Both of these latter observations have been interpreted as evidence that insulin deficiency is associated with a decrease in the proportion of hepatic ribosomes that exist as polyribosomes. It is obvious that such a decrease in the hepatic polyribosomal population could be responsible for the reduced hepatic protein synthesis seen in acute insulin deficiency, and in an effort to directly evaluate this possible relationship we have carried out the present study.

Rats were made diabetic by administration of alloxan, and we then characterized certain qualitative and quantitative changes in hepatic ultrastructure that occurred with acute insulin deficiency, as well as describing the manner in which these changes are reversed with insulin replacement. At each stage of insulin deficiency, or replacement, we have also correlated the changes that were observed in hepatic ultrastructure with the *in vitro* protein synthetic capacity of ribosomes obtained from the same livers. In general, a very good correlation existed between the severity of ultrastructural changes and measured decreases in *in vitro* hepatic protein synthesis. Among the most striking changes in hepatic ultrastructure that occurred in alloxan diabetes was a fragmentation and loss of the rough-surfaced endoplasmic reticulum (RER).¹ Since the RER constitutes the major polyribosomal pool of the normal hepatocyte, these results offer direct evidence that alloxan diabetes results in a decrease in the hepatic polyribosome population.

METHODS

I. Experimental protocol

Female Sprague-Dawley rats weighing between 180–200 g were used for all experiments. Animals were given Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.)

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¹Abbreviations used in this paper: RER, rough-surfaced endoplasmic reticulum, SER, smooth endoplasmic reticulum.

and tap water ad lib. except when otherwise indicated. Blood glucose levels were estimated by use of paper strips impregnated with glucose oxidase, and read by a Reflectance Meter² (5). All animals were placed into one of the following four treatment groups.

1) *Controls*. Animals in this group were fasted for 18 h, then injected with 0.5 ml saline into the tail vein. They were allowed to eat freely, and were killed at approximately noon 48 h later, at the same time as the alloxanized animals for whom they served as controls. Controls for insulin-treated animals were injected with saline at the same time the experimental animals received insulin.

2) *Fasted controls*. Some rats which were to serve as controls for alloxanized animals were submitted to a second fast, which took place the last 18 h of the 48 h experimental period. This group of controls was considered necessary since very sick alloxanized animals often did not consume the normal amount of food from 36 to 48 h after alloxanization.

3) *Alloxan treatment*. Rats were given alloxan (70 mg/kg body weight) by tail vein after an 18 h fast, and were killed 48 h after injection. Not all injections were technically perfect, and this may help explain the fact that blood glucose levels varied from 300 to 1200 mg/100 ml 48 h after the alloxan was administered. Most rats in this group were allowed to eat freely during the experimental period, but a few were forced to fast for the terminal 18 h of the 48 h experimental period.

4) *Insulin replacement*. In order to clearly document the effects of insulin replacement, rats were not selected for this part of the study unless they were very hyperglycemic (blood glucose levels > 800 mg/100 ml). Insulin administration was started 48 h after alloxan and animals were sacrificed 1, 2, 4, 6, and 24 h after insulin replacement was begun. In some instances insulin was not given, and the surviving rats of this group were killed 24 h later. For short-term treatment, rats received 1 U regular insulin (intraperitoneally) every 2 h. For 24 h insulin replacement, a three-step program was followed. During the initial 6 h period, animals received 1 U of regular insulin (intraperitoneally) every 2 h. At this point they received 4 U of lente insulin (subcutaneously). During the final 6 h they again received 1 U of regular insulin (intraperitoneally) every 2 h.

II. Measurement of in vitro protein synthesis

1) *Preparation of ribosomes*. Rat liver was placed in 50 ml of ice-cold Medium A (0.35 M sucrose, 0.004 M MgCl₂, 0.07 M KCl, 0.05 M Tris-HCl, pH 7.8, 0.0008 M dithiothreitol). The liver was blotted, weighed, and homogenized with six strokes at 1000 rpm in a Teflon pestle-ground glass homogenizer, using 2 vol. Medium B (0.25 M sucrose, 0.05 M Tris-HCl, pH 7.6, 0.005 M MgCl₂, 0.025 M KCl). The homogenate was centrifuged for 15 min at 10,000 *g* to prepare a postmitochondrial supernate. The supernate was decanted and 0.15 ml 10% sodium deoxycholate was added per ml of supernate to bring the final deoxycholate concentration to 1.5%. The mixture was then allowed to stand 20 min on ice. Most pellets were produced by centrifugation of the supernate for 2 h at 105,000 *g* in a Spinco L2-65B ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) Some samples were prepared by centrifuging through a discontinuous sucrose gradient with a 0.5 M sucrose layered over 2.0 M sucrose (0.05 M Tris-HCl, pH 7.6, 0.005 M MgCl₂, 0.025 M KCl). These samples

² Ames Co., Elkhart, Ind.

were spun for 8 h at 105,000 *g* and yielded about one-third as much RNA as the nongradient samples. Ribosomes prepared by centrifuging through gradients were considerably more active in carrying out protein synthesis than were ribosomes prepared in the usual fashion, but the activity difference between control and diabetic ribosomes remained when both were prepared through gradients.

2) *Incubation of ribosomes*. A detailed description of the method with which incorporation of [³H]phenylalanine into protein was measured has been published earlier (2). In general, a suspension of ribosomes of known RNA content was added to an incubation medium with a pH 5 fraction obtained from normal liver, amino acids, [³H]phenylalanine, GTP, ATP, phosphoenolpyruvate, and pyruvate kinase, with dithiothreitol used as a sulfhydryl stabilizer. Conditions of the incubation were such that the ribosomes were the rate-limiting factor for incorporation of amino acid into protein. Timed portions of the incubation sample were withdrawn and placed on filter papers, the protein precipitated by the addition of cold 10% TCA, and the disks washed to extract nonprotein radioactivity (6). The disks were then dried and counted in a liquid scintillation spectrometer. Ribosomal RNA was measured by the method of Schneider (7) and protein-synthetic activity was expressed as counts per minute of [³H]phenylalanine incorporated into protein per milligram RNA in the incubated sample after a 30 min incubation. Ribosomes from control, alloxan, and alloxan plus insulin-treated rats were always prepared and incubated concurrently, and experimental results were expressed as a percentage of the synthetic activity of the control tissue prepared and assayed the same day.

III. Preparation of tissue for electron microscopy

The rats used for study were stunned by a blow on the head and exsanguinated through the chest. Tiny pieces of tissue were taken from the right lobe of the liver and processed by one or more of the following procedures: (a) fixation for 1½ h at 4°C in 1% osmium tetroxide buffered at pH 7.0 with Millonig's phosphate buffer. This procedure was used for general tissue fixation; (b) fixation for 1½ h at 4°C in 1% osmium tetroxide buffered at pH 7.0 with 0.1 M veronal acetate buffer followed by 1 h postfixation at 22°C in 0.5% uranyl acetate buffered at pH 6.0 in 0.1 M veronal acetate buffer. This procedure selectively emphasized cytomembranes while it eliminated the staining of glycogen; and (c) fixation for 2 h at 22°C in 4% osmium tetroxide in distilled water, pH 7. This procedure particularly emphasized the presence of free, nonmembrane-bound polysomes. All specimens were rapidly dehydrated in a graded series of alcohols, followed by propylene oxide, and embedded in a mixture of Epon and Araldite plastic.

IV. Morphometric studies

Liver specimens were taken from four rats of each of the following categories for morphometric studies: (a) controls, (b) 48-h alloxan-treated rats with blood glucose levels > 800 mg/100 ml, and (c) 48-h alloxanized rats with blood glucose levels > 800 mg/100 ml which had been treated with insulin for 24 h. The liver specimens were processed (as in section IIIa) and prepared for morphometric analysis as described by Loud (8, 9). Thick sections from the surface layers of plastic-embedded blocks of liver were stained with toluidine blue and used for locating midzonal hepatocytes and for measuring the average cell size

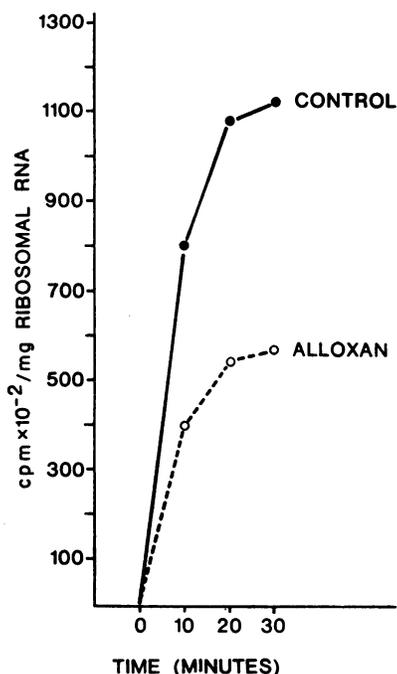


FIGURE 1 A comparison of the protein-synthetic activity (counts/minute per milligram ribosomal RNA) of a ribosomal preparation isolated from the liver of a control and an alloxan-treated rat.

of nucleated hepatic parenchymal cells. The specimen blocks were retrimmed after selection of the appropriate cells and thin, silver-gray sections were taken of three different blocks from each of the 12 animals and stained with uranyl acetate and Reynolds' lead citrate. Electron micrographs of very low magnification ($\times 3000$) were taken of sections of these 36 blocks and suitable areas of the micrograph plates were enlarged fivefold and printed on 11×14 in paper. Parallel sampling lines spaced 2.54 cm apart were drawn on each print and the percent volume occupied by mitochondria, glycogen, and lipid droplets was estimated from the fractional length of sampling lines covered by these cellular components (8-10). RER length per area cytoplasm (proportional to area RER per volume cytoplasm) was estimated by the following formula (8-10):

$$L = \frac{\pi \times C \times M}{2 \times 1000 \times l}$$

in which L represents the RER in micrometers per square micrometer cytoplasm; C , the number of intersections of RER profiles with the sampling lines; M , the total magnification of the photographic print; and l , the sum of the length of sampling lines measured in millimeters.

RESULTS

I. In vitro protein synthesis

Studies of the effect of insulin deficiency and replacement on the capacity of isolated hepatic ribosomes to incorporate labeled phenylalanine into protein in a cell-free amino acid incorporating system were carried out

on rats whose blood glucose levels were greater than 800 mg/100 ml 48 h after receiving alloxan. At this point the rats were either killed or started on insulin replacement and killed, 1, 2, 4, 6, or 24 h later. In several instances insulin was not replaced, and the surviving rats were also killed 24 h later. The effect of this degree of alloxan diabetes on the protein synthetic capacity of isolated hepatic ribosomes is illustrated in Fig. 1, in which ribosomes from the liver of a normal and diabetic rat are compared. In order to avoid the effects of day to day variations in the preparation and incubation of ribosomes, control and experimental ribosomes were always prepared and incubated concurrently. For the same reason, the experimental results were expressed as the percentage of the protein-synthetic activity of the control tissue prepared and assayed the same day. The results of 35 such comparisons are summarized in Fig. 2. These results indicate that the effect of alloxan diabetes is to reduce hepatic protein synthesis to approximately 55% of control values. No perceptible improvement in hepatic protein synthesis was observed during the first few hours after insulin replacement, but by 6 h some evidence of repair could be detected and hepatic protein synthesis had completely returned to values for control animals 24 h after beginning insulin. It should be noted that no improvement was seen in 24 h when insulin was not replaced.

II. Ultrastructural details

The ultrastructure of livers from rats with alloxan diabetes differed in many ways from normal. In an effort to clearly define these differences, we will describe the ultrastructural details under four general headings.

1) *Control rats.* Figs. 3, 4a, 4b, 5, and Table I illustrate a number of features of the normal hepatocyte relevant to this study. A complex of RER is shown occupying a well-defined area of the cytoplasm (Fig. 3).

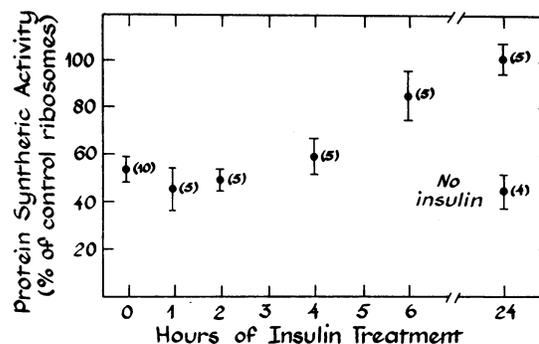


FIGURE 2 The effect of alloxan and insulin replacement on the ability of hepatic ribosomes to incorporate labeled phenylalanine into protein during in vitro incubations. Results are expressed as mean \pm SE and (n) refers to the number of animals.

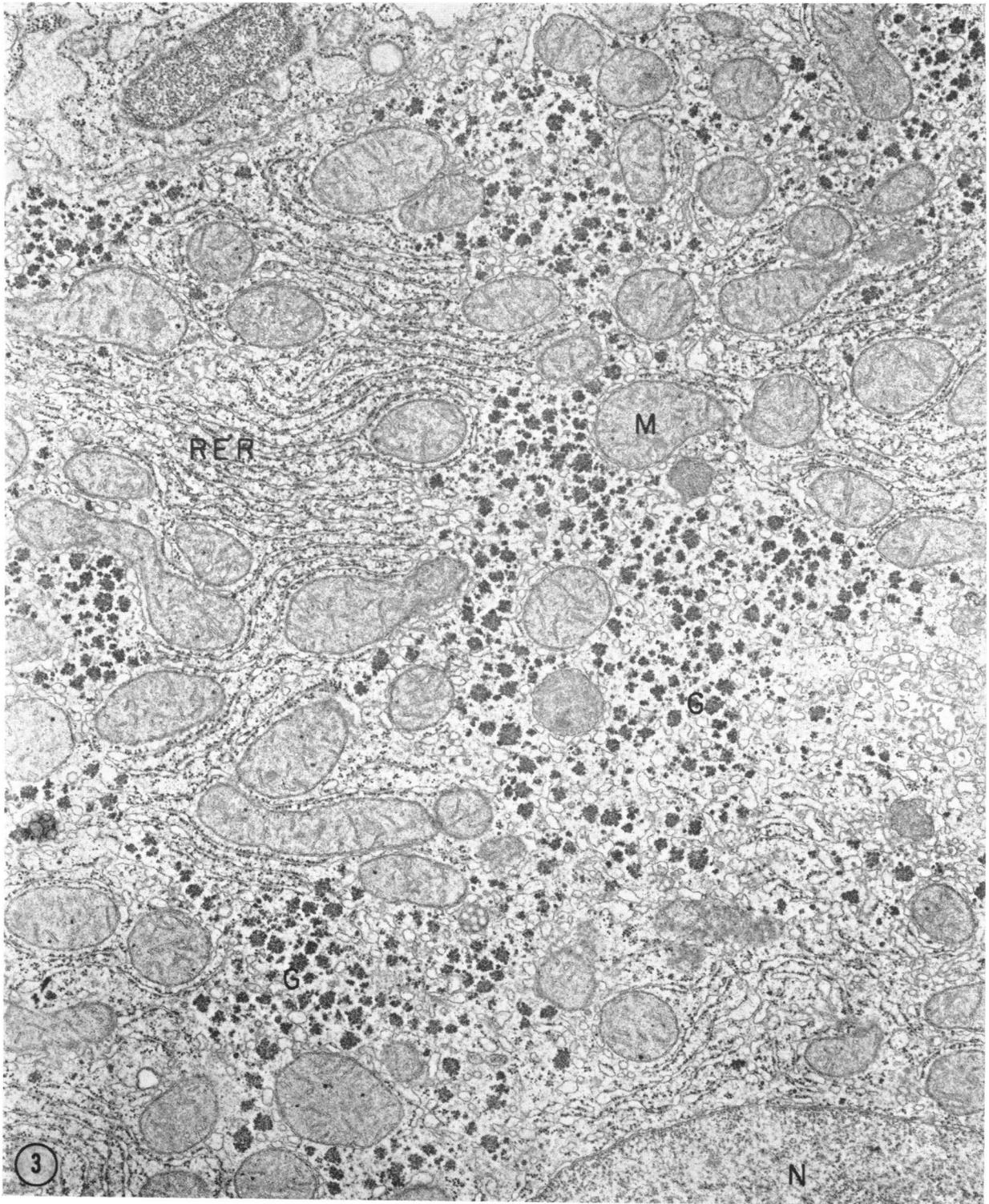


FIGURE 3 Low magnification view of hepatocyte showing organelle organization of normal cells. Rough endoplasmic reticulum (RER), mitochondria (M), glycogen (G), nucleus (N). (Magnification, $\times 21,800$.)

Typically, normal hepatocytes sectioned through a central area show several such zones. Each complex consists of up to 20 parallel cisternae of ribosome-studded endoplasmic reticulum membranes. Mitochondria may be randomly distributed throughout this zone. Tangentially cut membranes of endoplasmic reticulum are shown in Fig. 4a revealing the underlying shadow of the sectioned membrane and the characteristic spiral or rosette pattern of the membrane-bound polysome cut at this angle (11). Ribosomes also occur free in the cytosol of the normal hepatocyte as monoribosomes or in clusters, presumably as polyribosomes. Such clusters of polyribosomes may be located between the cisternal units of RER and in favorable sections, as in Fig. 4b, they can be differentiated from the membrane-associated polysomes cut tangentially, as in Fig. 4a.

In the normal hepatocyte, glycogen is found in specialized regions of the cytoplasm normally occupied by the loose framework of tubules and channels of the smooth endoplasmic reticulum (SER) (11). The general location of glycogen within the hepatocyte can be well seen in Fig. 3, in which glycogen is stained, but the particular relationship of glycogen to the SER is more clearly demonstrated in Fig. 5, in which glycogen is not stained. The typical fine structure of the mitochondria in a normal hepatocyte can be seen in Fig. 3.

Column 1 of Table I summarizes some relevant mor-

phometric data from normal midzonal hepatocytes of our group of rats. The figures represent an average of measurements taken from a total of 12 micrographs (i.e., one micrograph from each of three blocks from each of four different control animals). The values are roughly comparable with those reported by Loud (9) in fed rats of a different strain.

2) *Fasted rats.* Electron micrographs of livers from control rats fasted 18 h (Fig. 6) showed some minor alterations of the structures just described. In comparing Fig. 6 with Fig. 3 (normal control) it can be seen that fasting produces some apparent disorganization in the spatial relationships of the peripheral RER cisternae, which was independent of the orientation of the specimen during sectioning. Fig. 6 also demonstrates that an 18 h fast results in sufficient depletion of glycogen from the SER area to reveal the normal loosely arranged network of tubules of this complex organelle.

3) *Alloxanized rats.* The changes in rat hepatic ultrastructure that accompanied alloxan diabetes could be somewhat arbitrarily subdivided into three general categories, based upon the degree of variation from normal. These categories, designated as mild (1+), moderate (2+), and severe (3+), were also related in a general fashion to the degree of hyperglycemia, e.g., the higher the blood glucose, the more severe the change in hepatic ultrastructure.

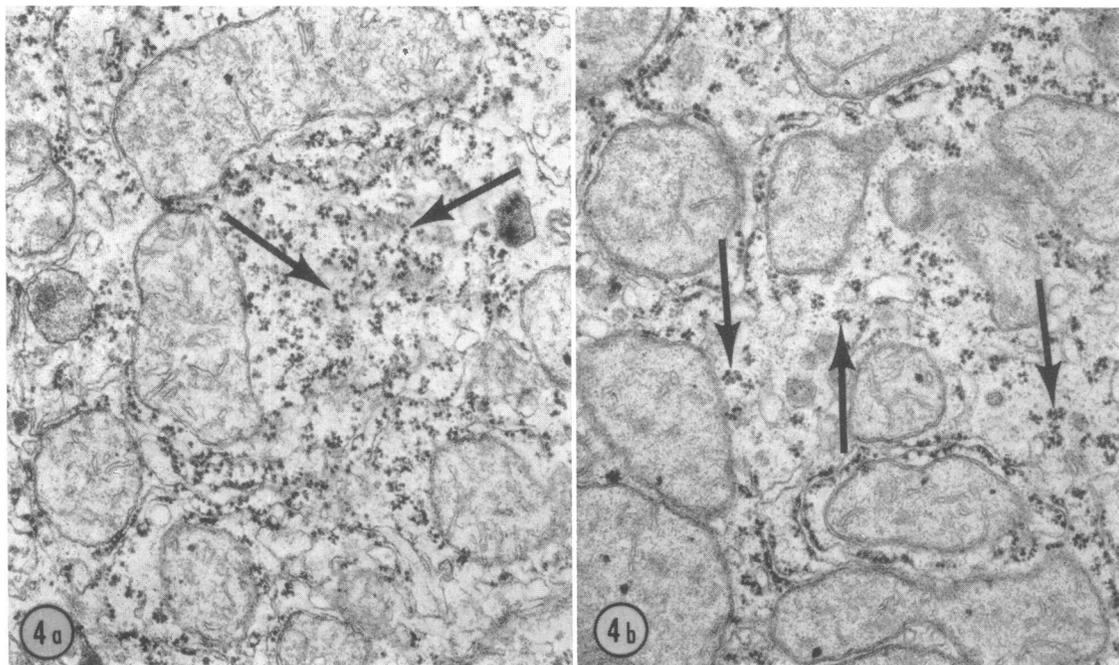


FIGURE 4 (a) Portion of normal hepatocyte in which a number of cisternae of the RER were cut tangentially demonstrating spiral pattern of membrane-bound polysomes (arrows). (Magnification, $\times 37,000$.) (b) Portion of normal hepatocyte demonstrating a number of free (nonmembrane bound) polysomes (arrows). (Magnification, $\times 37,000$.)

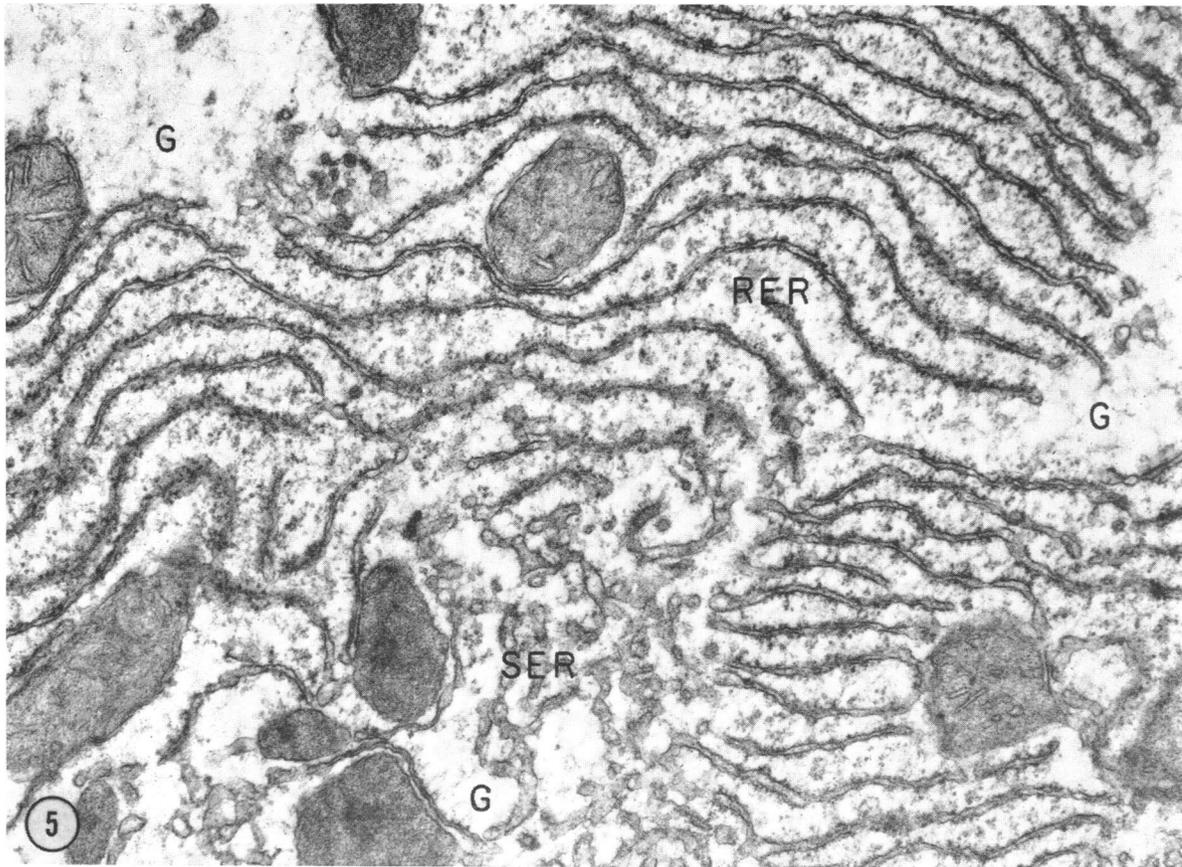


FIGURE 5 Normal hepatocyte postfixed in uranyl acetate to demonstrate cytoplasmic membranes. Note loose arrangement of smooth endoplasmic reticulum (SER) continuous with rough endoplasmic reticulum (RER). Glycogen (G) is not stained. (Magnification, $\times 37,000$.)

(a) *Mild diabetes (1+)*. The morphological characteristics of livers from rats whose blood glucose ranged from 300 to 500 mg/100 ml were qualitatively similar to those of control rats fasted for 18 h (Fig. 6)

in that glycogen was reduced in quantity and some disorganization of the RER was apparent.

(b) *Moderate diabetes (2+)*. The characteristic ultrastructural changes of livers from rats whose blood

TABLE I
*Morphometric Measurements of Cytoplasmic Components in Midzonal Hepatocytes**

	Control (n = 4)	Alloxan (n = 4)	Alloxan + insulin (n = 4)
		<i>Mean \pm SE</i>	
Area of RER, μm^2			
Volume of cytoplasm, μm^3	2.67 \pm 0.20	0.88 \pm 0.05‡	3.12 \pm 0.43
Percent cytoplasmic volume			
Mitochondria	15.25 \pm 1.38	22.55 \pm 0.61‡	20.85 \pm 2.06§
Lipid	0.20 \pm 0.09	18.65 \pm 4.92§	0.09 \pm 0.04
Glycogen	14.75 \pm 2.32	0.09 \pm 0.02‡	20.10 \pm 5.24

* These values are derived from the analysis of three micrographs from each of four rats in each group.

‡ $P < 0.001$ as compared with control values.

§ $P < 0.05$ as compared with control values.

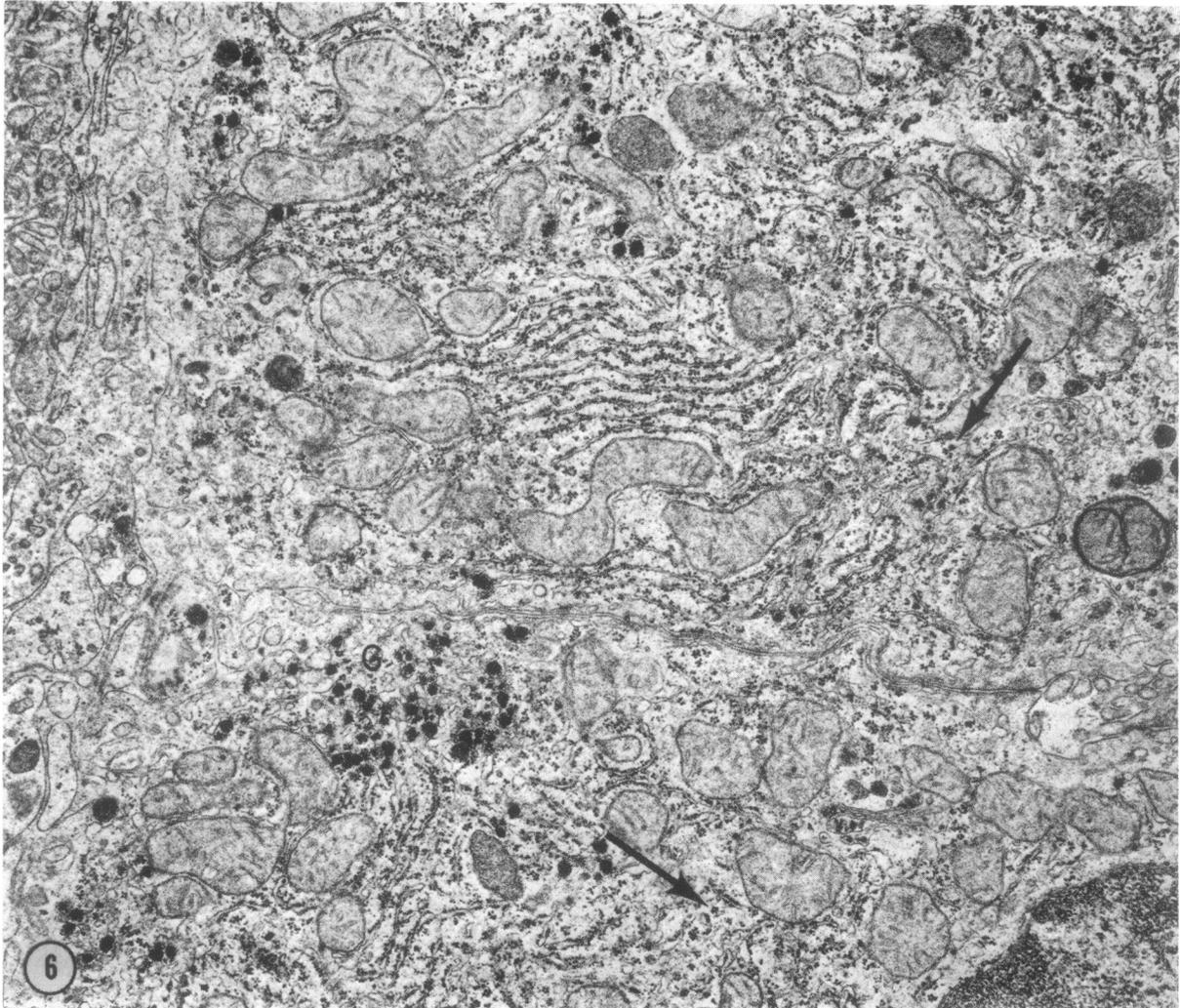


FIGURE 6 Low magnification view of hepatocyte from 18 h fasted rat. Note that glycogen (G) pool is reduced, and some disorganization of RER is apparent (arrows), but cytoplasm is otherwise normal in appearance. (Magnification, $\times 18,000$.)

glucose levels ranged from 400 to 800 mg/100 ml are illustrated in Fig. 7. It is apparent that the highly organized RER of the normal hepatocyte (Fig. 3) has been lost, and that the parallel profiles of ribosome-studded endoplasmic reticulum membranes have been replaced by what appeared to be remnants of RER.

(c) *Severe diabetes (3+)*. Hepatocytes from rats whose blood glucose exceeded 800 mg/100 ml showed the most striking ultrastructural changes (Figs. 8-10). The decrease of the RER appeared to be extreme in this group of animals; only isolated profiles of RER could still be seen (Figs. 8 and 9). This impression was confirmed by morphometric analysis of hepatocytes from these animals in which the area of RER per volume cytoplasm was found to be significantly ($P < 0.001$) reduced to roughly one-third the quantity found

in normal hepatocytes (Table I). Although actual counts were not made of free polyribosomes in these electron micrographs, there did not appear to be an equivalent loss of these structures and their relative abundance can be seen in Figs. 8 and 9. Accompanying the reduction of RER was a marked proliferation of SER membranes which took the form of tightly tangled, branching, and anastomosing channels (Figs. 8 and 10). Few particles of glycogen remained in these foci of SER or elsewhere in the cytoplasm. Concentric lamellar formations consisting of tightly packed lengths of membranes were seen with some regularity in these hepatocytes (Figs. 8 and 10).

Mitochondria and lipid droplets (Figs. 8 and 10), were greatly increased in size and represented major space-occupying organelles in the cytosol of hepatocytes

of alloxanized animals as seen in Table I. It should be noted that although the average cell size of alloxanized hepatocytes did not differ from that of control hepato-

cytes, there was considerable variation in size from specimen to specimen depending upon the relative volumes occupied by lipid and mitochondria. Measurements

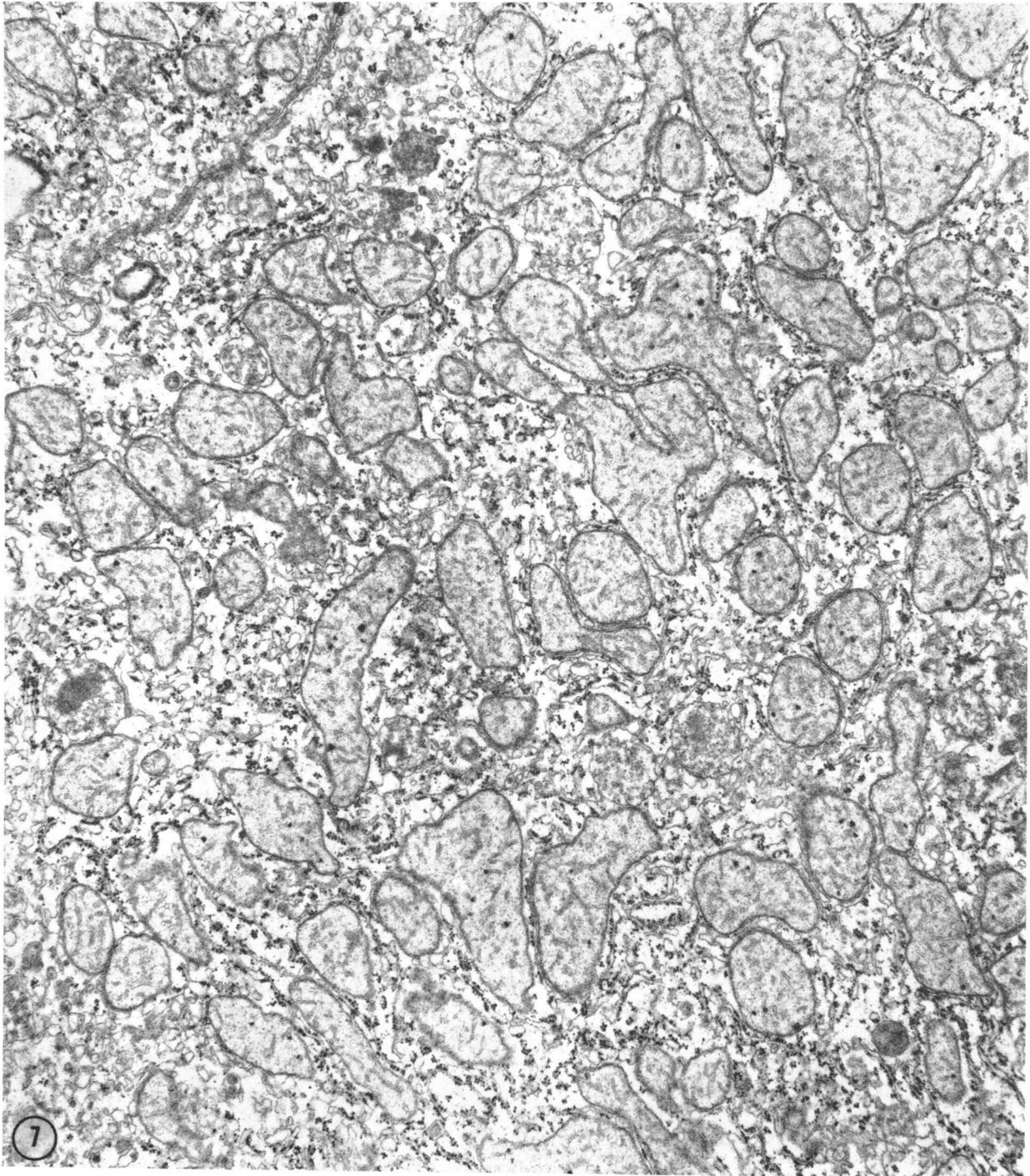


FIGURE 7 Low magnification view of hepatocyte of moderately ill alloxanized rat. Sections such as this were rated 2+ in Fig. 12, and were obtained from rats with blood glucose levels ranging from 400 to 800 mg/100 ml. Note the extensive fragmentation and reduction of the RER. Free polysomes are abundant. Glycogen is absent. (Magnification, $\times 21,800$.)

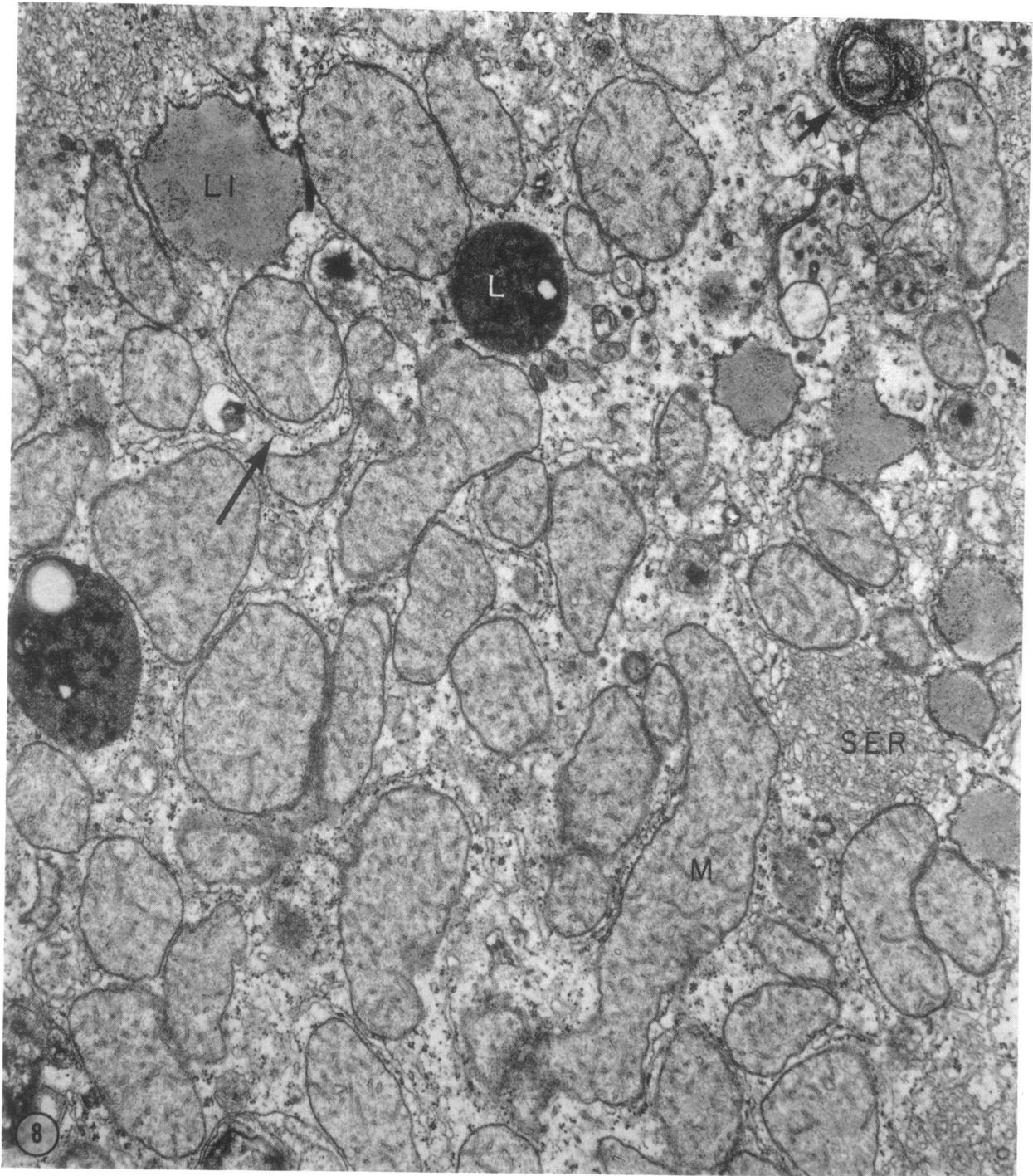


FIGURE 8 Low magnification view of hepatocyte of a severely ill alloxanized rat with a blood glucose level in excess of 1000 mg/100 ml. This specimen was rated 3+ in Fig. 12. Note that the RER is greatly reduced in amount. Occasional profiles of these membranes appear to encircle mitochondria (long arrow). Free polysomes are abundant. The smooth endoplasmic reticulum (SER) is highly developed. Mitochondria (M) are greatly increased in size. Lipid droplets (LI) and lysosomes (L) are prominent. The short arrow points to an abnormal formation of cytomembranes. (Magnification, $\times 21,800$.)

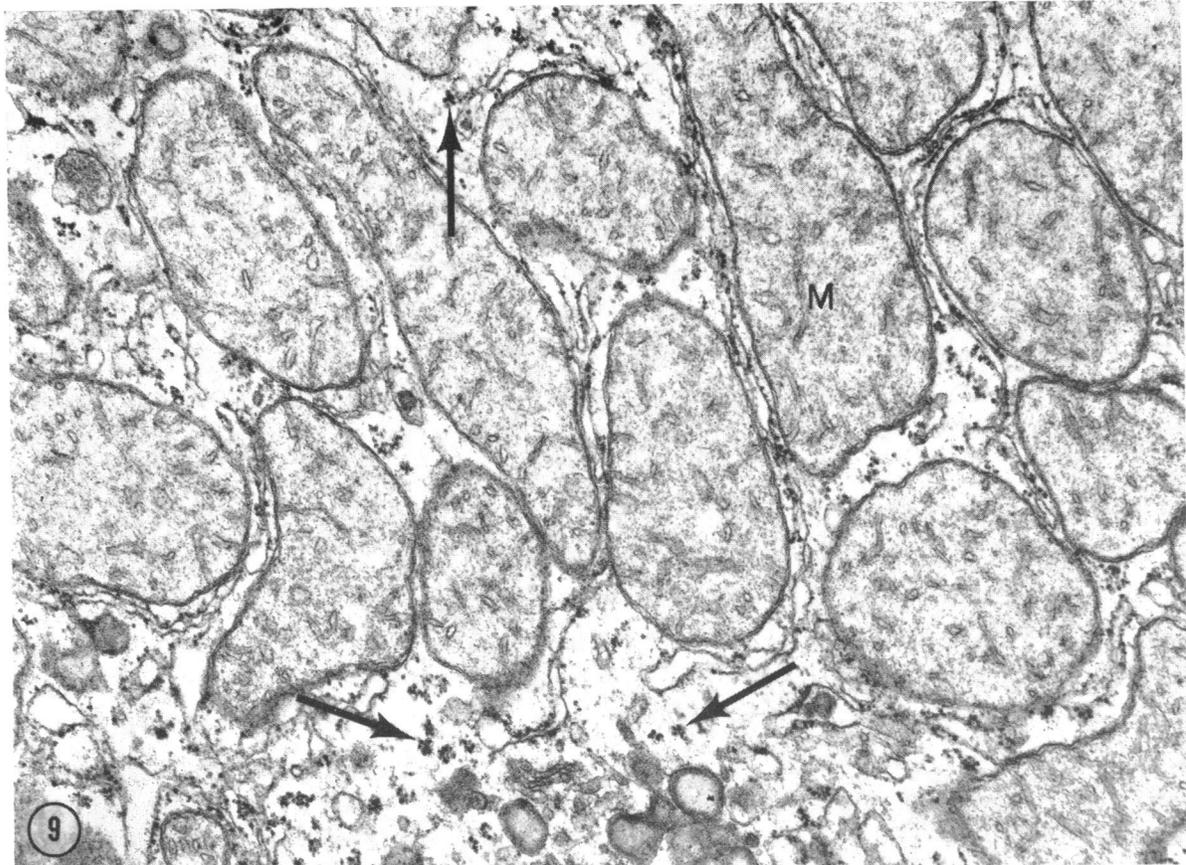


FIGURE 9 Portion of hepatocyte from severely ill rat (blood glucose > 1000 mg/100 ml), demonstrating scarcity of RER. Arrows point to free polysomes which remain abundant. Mitochondria (M) are abnormally large. (Magnification, $\times 37,000$.)

of size of alloxanized cells with large amounts of lipid and very enlarged mitochondria averaged 10% greater than control cells; the size of alloxanized cells with little lipid and little enlargement of mitochondria averaged 10% less than control cells. Since it was impossible to know the state of hydration of these cells at the time of fixation, no use was made of these figures in estimating RER in the cytoplasmic space.

d) *Insulin-treated rats.* Although insulin therapy ultimately reversed the ultrastructural alterations encountered in the liver of alloxanized rats, preliminary experiments had indicated that the rate at which recovery occurred was clearly dependent on the severity of diabetes at the onset of insulin administration. For this reason, rats chosen for critical evaluation of the benefits of insulin therapy after alloxanization had blood glucose levels greater than 800 mg/100 ml at the onset of treatment. As noted above, alloxan-induced hepatic ultrastructural changes at this level of hyperglycemia were consistently severe.

The most immediate effect of insulin was to stimulate glycogen deposition, and within 6 h after the onset of insulin administration glycogen was already so extensively associated with areas of SER so as to obscure the underlying membranous character of this site (Fig. 11). Although the RER appeared far from being repaired at this stage, all cells showed accumulations of polyribosomes and foci of reassembling RER. Mitochondria still appeared to be enlarged at this stage.

By 24 h of insulin replacement the hepatocyte ultrastructure of all animals, even those which previously had been close to death, showed striking signs of recovery (Fig. 12 and Table I). Glycogen deposits occupied a large proportion of the volume of each cell. Mitochondria were not yet of normal dimensions but somewhat reduced in size. Although the total quantity of RER present in the hepatocyte had returned to normal (Table I) in many areas (as illustrated in Fig. 12), membranes were not yet found in the parallel array characteristic of normal hepatocytes.



FIGURE 10 Portion of hepatocyte from same animal as in Fig. 8 postfixed with uranyl acetate to demonstrate cytoplasmic membranes. Note especially the tangled smooth endoplasmic reticulum (SER) and abnormal membrane formations (arrow). Lipid droplets (LI) are especially large in this section. (Magnification, $\times 37,000$.)

III. Correlation between changes in hepatocyte ultrastructure and decreases in hepatic protein-synthetic capacity

In 33 instances, liver from the same alloxanized animal was available for both measurement of protein synthesis and assessment of ultrastructural abnormality. Included within this group were animals who had received insulin replacement. The estimate of the decrease in hepatic protein synthesis was based upon comparing the activity of experimental ribosomes to control ribosomes, prepared and incubated on the same day. The percent change in activity was then correlated with an estimate of the severity of the changes in hepatocyte morphology that were observed in that same animal. Decisions as to degree of ultrastructural abnormality were made without knowledge of the animals' physiological state or of the measured change in protein-synthetic capacity, and each animal was assigned a value of from 0-3+ on the basis of criteria described earlier. The relationship between hepatic protein synthesis and morphological change for these 33 paired observations is seen in Fig. 12, which clearly indicates the high degree of correlation ($P < 0.001$) that existed between decreases in hepatic protein-synthetic capacity and relative degrees of abnormality of hepatic ultrastructure.

DISCUSSION

The results that have been presented confirm earlier studies which indicated that ribosomal preparations from rats with experimental diabetes mellitus were less capable of incorporating a radiolabeled amino acid into protein than were similar preparations obtained from normal rats (1, 2). These estimates of protein-synthetic activity do not tell us how experimental diabetes produces this decrease in protein synthesis, and it was in order to gain insight in this regard that led us to directly examine the changes in hepatic ultrastructure that occur in alloxan diabetes. Since there was no a priori reason to assume that changes in hepatic morphology are necessarily related to effects of experimental diabetes on hepatic protein synthesis, the extremely significant degree of correlation that was shown to exist (Fig. 13) between decreases in hepatic protein synthesis and ultrastructural abnormalities was reassuring. Obviously, this does not mean that the two variables are causally related, and it seems unlikely that changes in hepatic morphology such as loss of glycogen or accumulation of lipid are responsible for the decrease in hepatic protein synthesis. On the other hand, the observation that the RER is reduced to approximately one-third normal values in rats with acute alloxan diabetes seems to

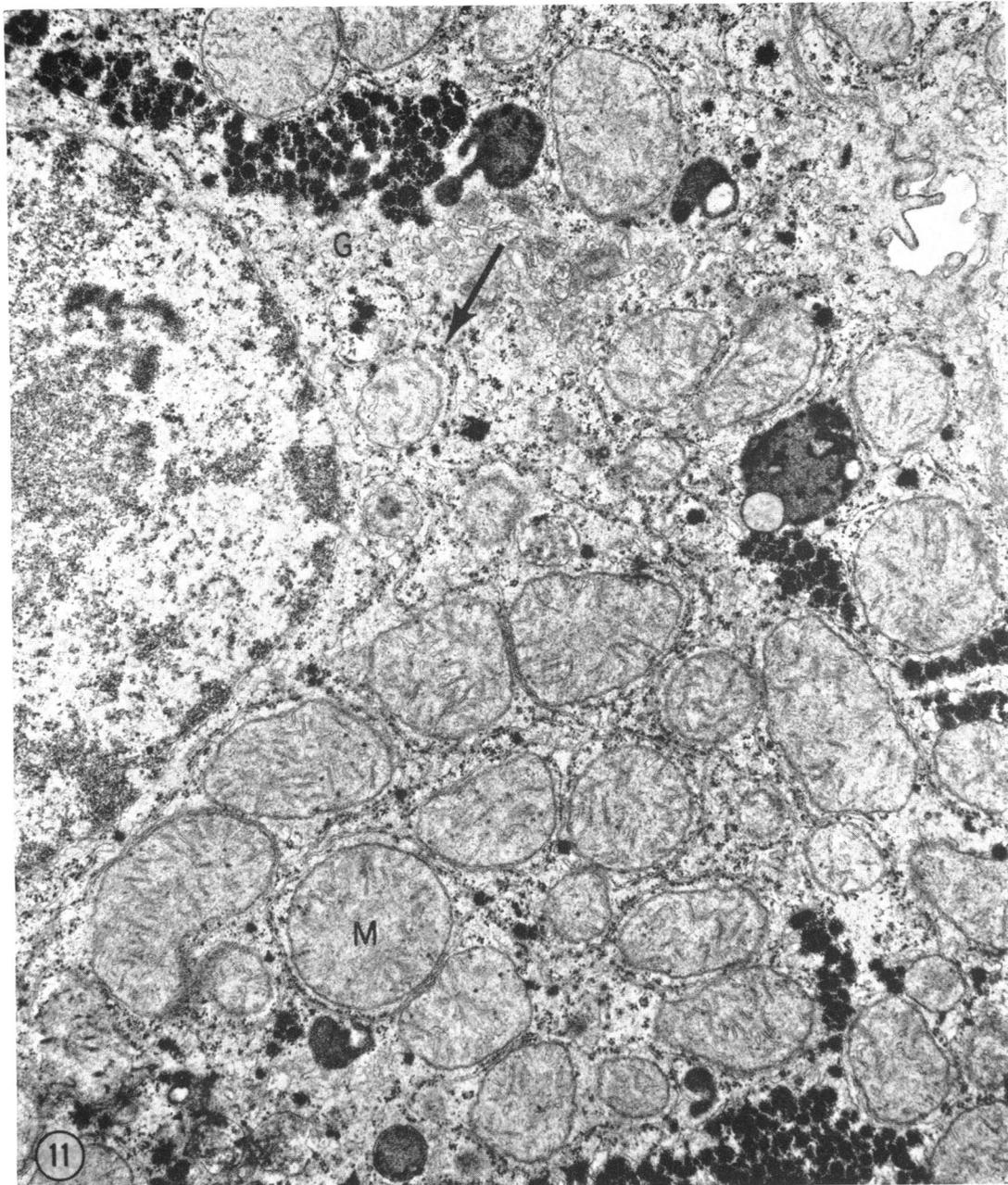


FIGURE 11 Low power view of hepatocyte from insulin-treated alloxanized rat. Blood glucose level before insulin replacement was in excess of 1000 mg/100 ml. Specimen was taken 6 h after onset of insulin administration. Note new glycogen (G) deposits and areas of reassembling RER (arrow). Mitochondria (M) are increased in size. (Magnification, $\times 21,800$.)

offer a reasonable explanation for the concomitant decrease in protein synthesis. Since the great majority of hepatocyte polyribosomes are normally bound to the endoplasmic reticulum membranes, the disruption, fragmentation, and eventual loss of the RER that occurs with alloxan diabetes would result in a marked loss of hepatic polyribosomes. It seems reasonable to assume

that as this process is going on that there would be a disaggregation of larger polyribosomes to smaller components. As a result, there would be a decrease in the proportion of hepatic ribosomal RNA present as large polyribosomes and an increase in the proportion present as smaller polyribosomes, monoribosomes, and ribosomal subunits. Since this latter group of ribosomal com-

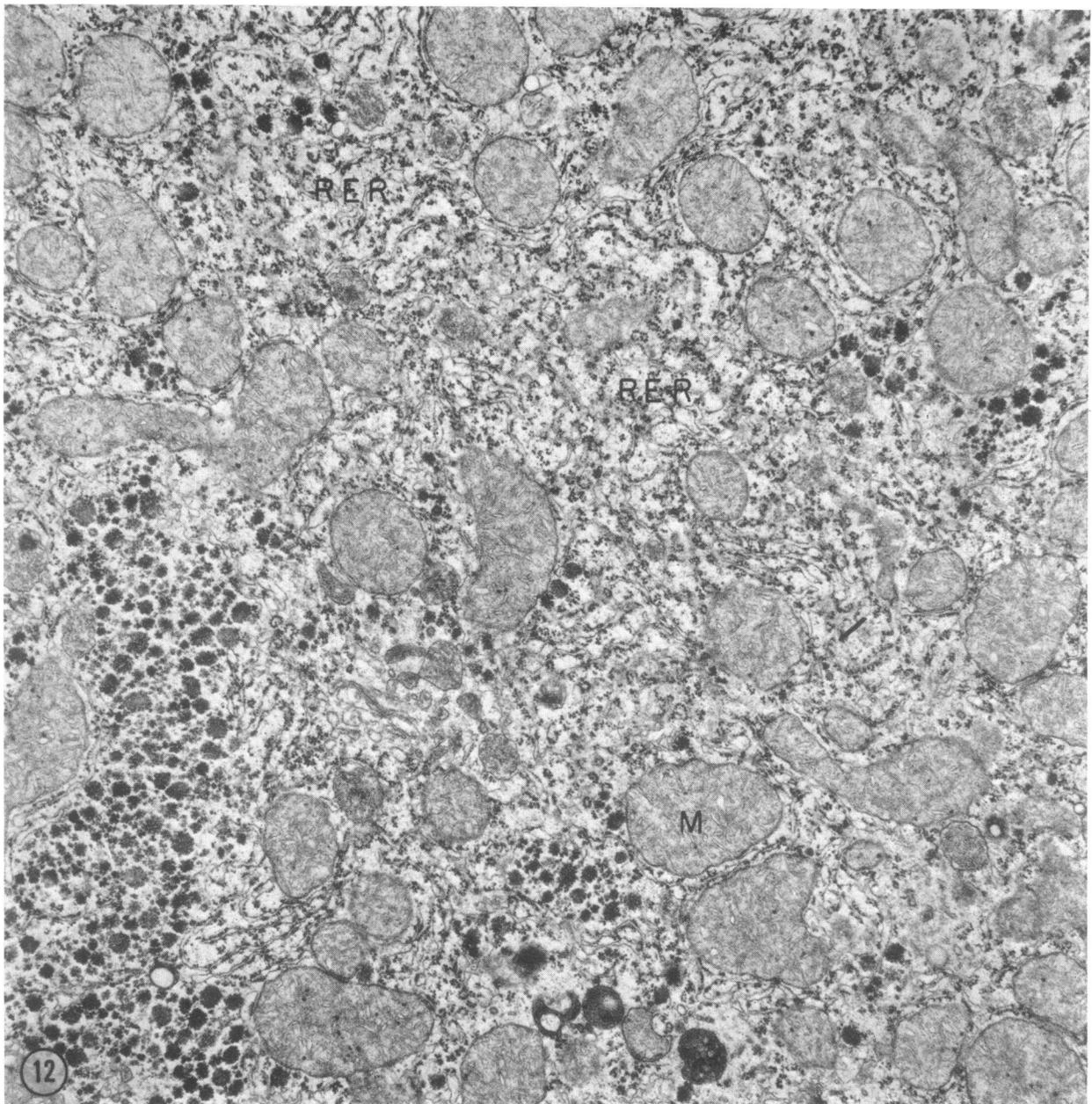


FIGURE 12 Low magnification view of hepatocyte from insulin-treated alloxanized rat. Blood glucose level before insulin replacement was > 1000 mg/100 ml. Specimen was taken 24 h after onset of insulin treatment. Note that the RER is abundant, although not organized into parallel stacks as in normal liver cells. Other cell organelles have normal appearance. (Magnification, $\times 21,800$.)

ponents is less active in protein synthesis, a decrease in protein synthesis per milligram of ribosomal RNA would result from this change in the hepatic ribosomal population. Thus, it appears that the decrease in hepatic protein synthesis that occurs with alloxan diabetes can result from the disruption and loss of RER, and the simplest explanation is that it is due to a decrease

in the proportion of hepatic ribosomes which are bound to messenger RNA and exist as polyribosomes. This hypothesis is also consistent with earlier (2), more indirect studies of the effect of experimental diabetes on hepatic protein synthesis, on polysome profiles (3, 4), and the binding affinity of ribosomes from diabetic animals for polyuridylic acid (3).

Obviously, the validity of conclusions as to the effect of experimental diabetes upon hepatic protein synthesis are contingent upon the premise that the observed changes are due to insulin deficiency and not pharmacological effects of the agents used to induce diabetes. In this regard, it appears that diabetes induced by either alloxan (2), anti-insulin serum (2), or streptozotocin³ produces equivalent reductions of hepatic protein synthesis. On the other hand, since disorganization and dissolution of the RER is a common result of hepatic injury caused by a wide variety of chemical agents and other stimuli (12), it is possible that the morphological changes we observed could be due to a direct effect of alloxan on the liver. In a similar fashion, the striking changes in mitochondrial size that we and others (13-15) have seen after the induction of alloxan diabetes could also result from a toxic effect of alloxan. However, all the abnormalities that we observed, even though extremely severe, were rapidly reversible after insulin administration. For example, within 6 h after insulin administration glycogen deposition was readily apparent and all cells showed accumulations of ribosomes and foci of reassembling RER. By 24 h of insulin replacement, the hepatocyte ultrastructure and morphologic characteristics approached normal. In contrast, continued deterioration of all aspects of hepatocyte ultrastructure occurred in alloxanized rats that did not receive insulin replacement. Since there is no reason to believe that insulin replacement would have any beneficial effect on hepatocyte damage caused directly by alloxan, it seems reasonable to assume that experimental diabetes, not the toxic effect of alloxan, is responsible for the changes in hepatic ultrastructure and protein synthesis that we have described.

Although Wool and colleagues (16) have reported somewhat analogous effects of alloxan diabetes on the *in vitro* protein-synthetic capacity of muscle ribosomes, there appears to be significant differences in the manner in which experimental diabetes modifies protein synthesis in the two tissues. In the first place, we have previously pointed out that there are differences in the way in which polyuridylic acid modifies protein synthesis in hepatic as compared with muscle ribosomes obtained from diabetic rats (3). Secondly, insulin replacement in alloxanized rats can return muscle protein synthesis to normal within minutes (16), in contrast to the number of hours it took to reverse changes we noted in hepatic ultrastructure and protein synthetic capacity. Thirdly, there appears to be a difference in the ribosomal population affected by experimental diabetes. Since the great majority of the ribosomes present in muscle are free in the cytosol, the reduced activity of muscle ribosomes resulting from diabetes must be due

³D. Peterson, unpublished observations.

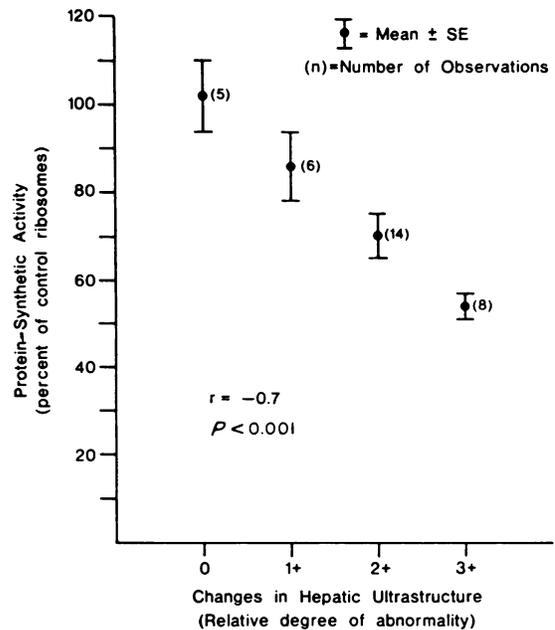


FIGURE 13 Correlation between alterations in hepatic ultrastructure with the protein-synthetic capacity of hepatic ribosomes. 0 change: normal organization of rat hepatocytes; 1+ change: slight disorganization of RER as in mild diabetic animals or in imperfectly repaired insulin-treated diabetic animals; 2+ change: disorganization, fragmentation and some loss of RER and depletion of glycogen as in moderately sick diabetic animals or in unrepaired insulin-treated diabetic animals; 3+ change: severe loss of RER; loss of glycogen; enlargement of mitochondria; increase in SER, increase in number of lipid droplets and lysosomes as in severely ill diabetic animals or unrepaired insulin-treated diabetic animals.

to an effect of insulin deficiency on these "free" ribosomes. In contrast, our results strongly suggest that changes in the "bound" ribosomal population are responsible for the defect in hepatic protein synthesis that resulted from alloxan diabetes.

The physiological significance of these differences is not immediately apparent, nor indeed is it clear as to the significance of the fact that alloxan diabetes seems to have effected the two ribosomal populations of the liver in a different fashion. One possible explanation for the latter phenomenon may be related to the fact that a major effect of diabetes on the liver is to increase the activity of several enzymes necessary for new glucose production (17). It seems possible that the changes observed in hepatocytes of rats with experimental diabetes may be viewed as a reflection of this process. Glycogen depletion in the diabetic animal is extensive. The amount of RER is drastically reduced, suggesting a curtailment in the synthesis of plasma albumin and other export proteins not essential for glucose production. On the other hand, there is extensive devel-

opment of the SER. The association of glucose-6-phosphatase with SER is now well established (18, 19), and it is possible that the observed increase in development of this organelle in the insulin-deficient animal is related to the recognized physiological need for this gluconeogenic enzyme. Obviously, the answer to these various questions will come only from further experiments. The issues are raised here primarily to emphasize the fact that significant and selective changes in the protein-synthetic activities of liver and muscle do occur as a result of experimental diabetes, and that we currently have very little insight as to the physiological significance of these changes.

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