PROTEIN DEFICIENCY IN RHESUS MONKEYS

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Protein malnutrition and kwashiorkor in children remain major nutritional problems in many tropical and subtropical countries.[1] Several experiments designed to produce a deficiency state simulating kwashiorkor have been performed in rats,[2-6] mice,[7] pigs,[8] rabbits[9] and monkeys.[10-13] Such experiments have been based predominantly on feeding either *ad libitum* or by intragastric tube diets deficient in protein or individual amino acids. In rodents, atrophy of the exocrine glands generally has been relatively slight, and the similarity to kwashiorkor has often been based predominantly on the existence of periportal fatty change in the liver. This is especially true when the diets were given *ad libitum*. Ramalingaswami, Deo and Sood[12,13] demonstrated, however, that many of the extrahepatic changes of kwashiorkor can be simulated by feeding monkeys a low protein diet with adequate calories. The present study was undertaken to compare further the systemic abnormalities produced by protein deficiency in monkeys with the lesions seen in rats and human subjects. The present report deals primarily with subcellular changes observed in the liver, pancreas, myocardium and striated muscle, and also with selected biochemical parameters.

MATERIAL AND METHODS

Eight female rhesus monkeys, weighing between 2,100 and 3,000 gm and approximately 1 to 2 years old, were studied. The animals were kept in individual cages, and preconditioned by feeding Purina® Monkey Chow for 3 months. They were divided into a control group and a protein deficient group as shown in Table I. The control group included 2 monkeys fed monkey chow *ad libitum*, one monkey fed a high protein diet which was isocaloric to the protein-deficient animals, and one monkey starved for 1 week. The protein deficient group was fed a 2 per cent casein diet for 4 weeks, at which time 2 monkeys were transferred to a protein-free diet for 2 to 4 weeks. The composition of the various diets based on 100 calories per kg body weight per day[12] is given in Table II. The diets were diluted with water to a suitable consistency and fed by intragastric tube in equally divided portions twice daily. The duration of diets and times of biopsies are also given in Table I.

Biopsies were carried out under light intravenous Pentothal® anesthesia at a dose

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TABLE I

DIETARY GROUPS AND SCHEDULE OF BIOPSY AND SACRIFICE

Monkey	Diets	Duration (weeks)	Weight (gm)		Weeks								
			Initial	At sacrifice	0	1	2	3	4	5	6	7	8
1	Monkey chow (*ad lib*)	7	2700	3050	B		B						S
2	Monkey chow (*ad lib*)	1	3000	3050		S							
3	20% Casein (isocaloric) *	8	2225	2450	B								S
4	Starvation	1	3000	2250			S						
5	2% Casein	7	2225	2200	B		B					S	
6	2% Casein	8	2235	2100	B				B				S
7	2% Casein and protein free	4 2	2290	2275	B		B				S		
8	2% Casein and protein free	4 4	2125	1850	B				B				S

B, biopsy; S, sacrifice.

* Isocaloric to protein-deficient diets (monkeys 5–8).

of 25 mg per kg body weight. With the exception of monkey 1 (control) which was sacrificed after a 14-hour fast, biopsy was carried out and animals were sacrificed 2 hours after the morning feedings and within 15 minutes after the induction of anesthesia. At sacrifice, specimens of selected organs were taken with minimal bleeding.

Tissues for electron microscopy were fixed in osmium tetroxide, buffered with *s*-collidine at pH 7.4, dehydrated in a graded series of alcohols and embedded in Epon 812 and Araldite. Sections were double stained with Karnovsky's lead hydroxide [14] and uranium, and were viewed with an RCA 3-B electron microscope. For orientation, adjacent semi-thin sections were stained with azure A in $NaHCO_3$. All organs were examined by light microscopy following formalin fixation and embedding in paraffin. Sections of liver and pancreas were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), diastase-PAS, Gordon-Sweet, Gomori's trichrome, phosphotungstic acid hematoxylin (PTAH) and methyl green pyronine stains. Striated muscle and myocardium were stained with H&E and PTAH. Fat was demonstrated by oil red O on frozen sections.

TABLE II

COMPOSITION OF DIETS

Constituents	Isocaloric high protein	Low protein	Protein free
Casein	20%	2%	0%
Sucrose	62%	80%	82%
Mazola® oil	10%	10%	10%
Salt mixture (U.S.P.XIV)	4%	4%	4%
Vitamin mixture (Choline—30 mg per gm diet)	4%	4%	4%

Total calories per day—100 per kg body weight.

At sacrifice, portions of the liver were assayed for lipids by ethanol-ether extraction, RNA and DNA by pentose analysis,[15] total proteins by the Lowry method,[16] and glycogen with the anthrone reagent.[17] Pancreatic tissues were also assayed for DNA, RNA and total proteins by similar methods. Blood for serum electrophoresis was taken at 2-week intervals.

RESULTS

General Findings

The protein deficient animals lost up to 13 per cent of their weight while the isocaloric controls and those fed monkey chow gained from 9 to 11 per cent (Table I). The hair in the deficient animals became coarse and sparse and the stools became soft, but frank diarrhea was not observed. Edema of the eyelids was observed only in 1 animal at the end of the observation period. In all the deficient animals, serum albumin decreased, and gamma globulins increased. There were no consistent alterations in the other globulins (Text-fig. 1). Differences between protein free and low protein animals were quantitative and not qualitative.

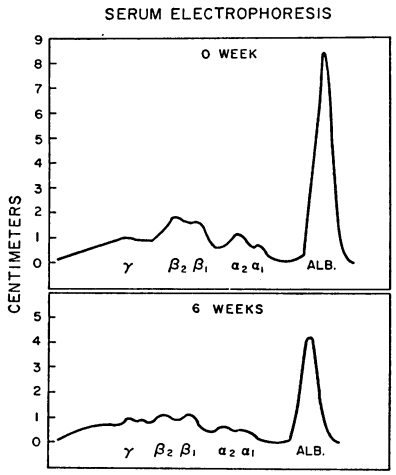
Liver

LIGHT MICROSCOPY

Periportal fatty metamorphosis was seen as early as 4 weeks (Fig. 1) and progressed moderately during the experiment (Fig. 2). Glycogen was abundant in experimental animals and in controls (except in the starved control, monkey 4). Cytoplasmic basophilia (pyroninophilia) was slightly decreased.

ELECTRON MICROSCOPY

Normal Monkeys (#1, 2 and 3). By electron microscopy the monkey liver, compared to the rat,[18] showed a relative paucity of cytoplasmic organelles (Fig. 3). Mitochondria and rough endoplasmic reticulum (ER) were sparse and were often associated spatially in the cytoplasm, particularly near the plasma membrane or opposite the bile canaliculi.



TEXT-FIG. 1. Monkey 8. Serum electrophoresis. The initial pattern (0 week) is representative of the normal while the protein deficient (6 weeks) pattern is typical of the deficient group. The albumin fraction consistently decreased while the gamma globulin increased.

The parallel stacks of rough ER were less abundant than in the rat. Free polyribosomes were generally seen near the rough ER. The smooth ER was well developed and generally intimately associated with glycogen particles. The nucleus was essentially similar to that in the rat with the exception that nucleus of the monkey liver cell frequently contained granular aggregates (measuring approximately 0.7μ or less), probably interchromatin granules. The 14-hour fasted control showed abundant smooth ER and loss of glycogen. The isocaloric control showed findings similar to those in the control fed *ad libitum.*

Protein Deficient Monkeys (#5, 6, 7 and 8). Apart from the accumulation of lipid bodies, the ultramicroscopic changes in the protein deficient animals were slight (Fig. 4). Lipid bodies varied in size and were generally electron-lucent and membrane limited. Intimate association between lipid bodies and mitochondria was not seen. The ER, both rough and smooth, was only slightly decreased. The cisternae of the rough ER showed slight focal dilatation. Smooth ER was reduced in amount and associated with glycogen particles but large glycogen-rich areas were devoid of such membranes. The free ribosomes were also decreased in amount, often markedly. Foci of cytoplasmic necrosis were absent and there was no apparent change in the mitochondria, nuclei or or nucleoli.

Pancreas

LIGHT MICROSCOPY

Compared to the normal (Fig. 5) the pancreas in protein deficient animals was markedly atrophic at 6 to 8 weeks (Fig. 6) and showed severe loss of zymogen granules and of cytoplasmic basophilia. Focal cytoplasmic vacuolar degeneration was observed and was often associated with dense irregular eosinophilic or basophilic bodies which stained in positive manner with diastase-PAS, and contained minimal amounts of fat.

ELECTRON MICROSCOPY

Normal Monkeys (#1, 2 and 3). The ultrastructure of the normal monkey pancreatic acini (Fig. 7) was quite similar to that described in other species.[19–21] The rough ER was well developed and prominent, with numerous parallel profiles in the basal portion and with transition into an irregular arrangement towards the apex. Free polyribosomes were seen occasionally. In the basal portion, widely dilated cisternae appeared as sinuses or vesicles depending on the plane of section. Numerous zymogen and few prozymogen granules were apparent in the apical portion of the cell. The lumens of the acini contained a dense fibrillar material approaching the electron density of zymogen granules (Figs. 7 and 13).

Protein Deficient Monkeys (#5, 6, 7 and 8). In the protein deficient monkeys, the acinar cells (Fig. 8) were markedly atrophic with disorganization or loss of the normal cell architecture, particularly of the rough ER. Even in the basal portions (Figs. 8 and 9), the rough ER was attenuated, irregular and focally dilated, with a decrease in attached ribosomes. The intermembranous cytoplasmic matrix was markedly increased in amount. In some cells there was relative increase in the number of free polyribosomes, often with a spiral pattern (Fig. 10).

There was an apparent loss of normal appearing zymogen and prozymogen granules which were replaced at the lumen aspect of the cell by many irregular vacuolar bodies of varying size (Figs. 11 and 12), with occasional transition between the smaller vacuoles and Golgi vesicles (Fig. 11). The vacuoles generally contained a granular, faintly electron-dense material, often mixed with highly osmiophilic precipitates and layers of fibrillar substance attached to their inner surface. In the apical portions, continuity of vacuoles with the acinar lumen suggested a secretory process (Fig. 12). The finely granular lumen content was similar to that in the vacuoles and contrasted markedly with the osmiophilic homogeneous secretion observed in the normal control (Fig. 13).

Multiple pleomorphic foci of cytoplasmic necrosis were observed frequently, often involving the greater portion of the cytoplasm (Figs. 8, 9 and 14). These areas were either membrane limited or lay free in the cytoplasm (Fig. 14). They contained dense osmiophilic precipitates which appeared either as fine granular deposits or as massive aggregates. Often they also included individual cell organelles such as mitochondria, prozymogen granules or ER and highly dense globular bodies, probably lipid. Arrays of fibrillar substance were also seen and a few showed a myelin-like pattern. In the surrounding intact cytoplasm, similar fibrillar arrays were present.

Occasional membrane limited lipid bodies appeared in the nuclei. Although coarsening and fragmentation of the nucleoloneme was observed, similar lesions occasionally appeared in controls.

Comparable changes in the rough ER or cytoplasmic necrosis were not encountered in the controls (monkeys #1, 2, 3 and 4).

Heart

LIGHT MICROSCOPY

The myocardial fibers were atrophic. There were no areas of coagulation necrosis or fatty change.

ELECTRON MICROSCOPY

Normal Monkeys (#1, 2 and 3). The normal monkey myocardium was essentially similar to that described in other species.[22,23]

Protein Deficient Monkeys (#5, 6, 7 and 8). By electron microscopy, patchy defects were noted in the myocardial fibers (Fig. 15). These areas were deficient in myofilaments and the cytoplasm was finely granular and contained remnants of membranes or dilated vesicles. The mitochrondria were far fewer in number than normal and some showed a 'moth-eaten' appearance suggesting lysis of their limiting membranes.

These defects involved both the paranuclear zone and myofibrils. There were no comparable lesions seen in the controls fed *ad libitum,* or by tube, or those starved.

Striated Muscle

LIGHT MICROSCOPY

Myofibers were atrophic, with decrease of cross-sectional area to almost half the normal. There were no other significant abnormalities.

ELECTRON MICROSCOPY

Normal Monkeys (#1, 2 and 3). The normal striated muscle of the monkey was essentially similar to that in other species.[24,25]

Protein Deficient Monkeys (#5, 6, 7 and 8). The myofibers were often narrow, and a loss of myofilaments of varying degree was seen (Fig. 16). There was focal loss within one or several sarcomeres with or without disruption of the Z membranes. Often a single myofiber was affected for the length of several sarcomeres or units of adjacent myofibers were involved. The areas deficient in myofibrils were occupied by a finely granular substance with remnants of sarco-tubular systems and enlarged and irregular mitochondria.

Other Organs

By light microscopy, there was atrophy of the salivary glands and lymphoid tissues. The gastrointestinal mucosa was normal or slightly atrophic.

Biochemical Alterations

As indicated in Table III, the liver RNA was normal or slightly decreased while the total proteins were decreased by one-half. The degree of increase in the lipids was not as great as anticipated from the light microscopic appearance. The glycogen control showed no distinct pattern, though it tended to be increased in the protein deficient group. The control fed *ad libitum* and fasted overnight showed a marked decrease in glycogen. The decrease in the amounts of pancreatic RNA and total protein was more prominent and more consistent than were corresponding values in liver.

Discussion

By light microscopy the lesions in the present experiments were similar to those reported by Ramalingaswami and co-workers [12,13] and qualitatively similar to those described in kwashiorkor.[26] Although the initial weights of the monkeys in the present study were similar to those used

TABLE III

CHANGES IN PROTEINS, RNA, GLYCOGEN AND LIPIDS IN LIVER AND PANCREAS

Monkey	Diet	*Liver* RNA	*Liver* Protein (mg/mg DNA)	*Liver* Glycogen	*Liver* Lipids (mg/gm)	*Pancreas* RNA	*Pancreas* Protein (mg/mg DNA)
1	Monkey chow	2.7	70	5*	34	3.6	46
2	Monkey chow	3.0	65	18	37	5.0	44
3	20% Casein	2.3	51	22	31	3.6	42
4	Starvation	2.7	58	2	88	3.0	27
5	2% Casein	2.5	53	18	37	2.2	26
6	2% Casein	2.8	40	25	51	1.7	17
7	2% Casein and protein free	1.9	40	19	80	1.6	20
8	2% Casein and protein free	2.5	36	28	55	1.8	20

* Fasted overnight, the rest sacrificed 2 hours post-prandial.

by Ramalingaswami and co-workers, 2 monkeys reached menarche during the course of the experiments. It was concluded that the monkeys were older than the 6 months originally planned although they had been the youngest the dealer could supply. Secondly, the long conditioning of the animals on an adequate diet may have made it more difficult to produce deficiency during the duration of the present experiment. The older age and long preconditioning may thus explain our failure to produce as severe fatty change in the liver as seen by the Indian group. Accordingly, our experiment would correspond more closely to juvenile kwashiorkor [26] in man than in weaned infants. On the other hand, these factors did not appear to affect the pancreas to the same degree as the liver since the pancreatic changes were more marked than those seen in rodents.[7,27]

Liver

Periportal fatty metamorphosis with progression to panlobular fat accumulation is observed in kwashiorkor,[26] or in experimental protein or amino acid deficiency.[2-13] Apart from fatty change, the ultrastructural alterations in our animals were minimal and contrasted with the marked changes in the ER, mitochondria and nuclei in protein deficient rats.[5,27] There was slight attenuation of the rough ER, but this change was far less than in the pancreas. The hepatic alterations resembled those described in kwashiorkor by Camain, Rouiller and Dupin [28] but the increased prominence of the cell membranes and sinusoidal recesses between hepatocytes [29] were not seen. However, the possibility that more severe changes might have been produced in younger monkeys cannot be excluded. The hyperplasia of the smooth ER prominent in the 14-hour fasted control suggested a barbiturate effect.

Pancreas

The pancreas appeared to be the most severely altered organ in the monkey. The extent and degree of disorganization of the rough ER and the cytoplasmic necrosis have not been reported in previous studies in rats and rabbits. Weisblum, Herman and Fitzgerald [30] described widening of the spaces between membranes in protein deficient rats, while Svoboda, Grady and Higginson, and Lazarus and Volk found no significant alteration in the rough ER in rats [27] or rabbits.[31] In the rabbit, foci of cytoplasmic necrosis were shown by Lazarus and Volk [31] to contain acid phosphatase, indicating their lysosomal nature. In their reports, the degree of cytoplasmic necrosis appeared to be less than in the monkey. Cytoplasmic degradation or necrosis is a nonspecific cellular response produced by several stimuli [32]; it is of interest that it does not appear to

be a feature of protein deficiency in the rat, in either the liver or pancreas.

The morphologic features in the pancreas were not unique to protein deficiency in the monkey. Similar changes in the rough ER associated with cytoplasmic necrosis have been observed in rats given agents that interfere with protein synthesis such as ethionine,[33,34] DL-homocystine,[35] and azaserine.[36] Similar alterations in rats are caused by triparanol and diethanolamine [37] which interfere with lipid metabolism.

By light microscopy loss of zymogen granules is observed consistently in kwashiorkor and in experimental protein deficiency. The secretion of the vacuolar body content shown by electron microscopy (Fig. 12) indicated that the vacuolar contents were analogous to degenerating prozymogen granules, and suggested secretion of an enzyme-deficient pancreatic juice. Decreased protein content of such prozymogen granules is produced with beta-3-thienylalanine [38] which causes morphologically similar alterations in zymogen secretion. In kwashiorkor, pancreatic enzymes have been described as deficient [39] at a very early stage of the disease before fatty change of the liver is evident.[40]

Ultrastructural comparison of pancreatic alterations in monkeys with those in man (kwashiorkor) cannot be made since electron microscopic studies in the latter have not been done. On the other hand, the light microscopic lesions shown were very similar to those described in man. Davies [41] has postulated that the pancreas is the organ primarily affected in kwashiorkor, since in many cases symptoms of pancreatic insufficiency are the first manifestations, and pancreatic atrophy may be more prominent than hepatic steatosis in subclinical cases.[42] Davies also indicated that the liver changes could be reversed without clinical improvement. In addition, protein malnutrition has also been implicated in pancreatic atrophy and fibrosis in young individuals in the tropics where the usual causes of pancreatitis could not be elicited.[43,44] In monkeys deprived of protein for longer periods than in this study, Ramalingaswami and coworkers,[12,13] described bulky stools with pancreatic atrophy.

In the monkey, the degree of atrophy, the extensive cytoplasmic necrosis, and the non-membrane limited characteristic lesions, suggested a true cellular injury rather than merely an adaptive response. On the other hand, similar changes, except the non-membrane limited cytoplasmic necrosis, have been shown to be readily reversible in rats [30] and bats.[45]

Myocardium and Striated Muscle

Atrophy of myocardial fibers [46,47] and striated muscle,[48] and electrocardiographic abnormalities have been reported in kwashiorkor and experimental protein deficiency. In protein deficient monkeys, extensive focal coagulation or rarefaction of the fibers has been described by

Chauhan, Nayak and Ramalingaswami.[47] The myocardial defects in the present subjects were focal and probably represented an early stage of the lesions described by Chauhan and co-workers. While loss of myofilaments has been seen in chronic protein deficiency in rats,[27] the changes were slight despite prolonged, severe deficiency in young animals. Loss of myofibrils, with or without mitochondrial alteration, is a nonspecific injury which is seen as a primary lesion in papain injection,[49] or as a part of cellular necrosis in other forms of injury including ischemia,[50,51] electrolyte deficiencies [52,53] or Plasmocid toxicity.[54]

The lysis of myofibrils in the striated muscle (sartorius) was very prominent and does not appear to have been described in protein deficiency. Atrophy of striated muscles in kwashiorkor and experimental protein deficiency [46–48] is well known, and the changes described in this report may correspond to it. A similar lesion has been observed in the rat diaphragm [54] with Plasmocid poisoning and some loss of myofilaments occurs in starvation.[55]

The muscle lesions may reflect the selective loss of muscle proteins in experimental [56] and human malnutrition.[57]

Biochemical Studies

The changes in ribonucleic acids, total proteins and lipids in the liver in kwashiorkor and experimental protein deficiency, are well known [58–61] and our findings were consistent with these. The proportionate decrease in RNA and proteins in the pancreas correlated with the marked severity of the lesions in that organ.

Conclusions

Uncomplicated protein deficiency in rhesus monkeys was studied. Ultrastructural changes in the liver, pancreas, myocardium and striated muscle were described in detail. The light microscopic features and the biochemical alterations in serum and liver were similar in many respects to those described in kwashiorkor.

The pancreas appeared to be the most severely damaged organ although lesions occurred in cardiac and striated muscle. The changes in the rough endoplasmic reticulum of the pancreas correlated well with the decrease in ribonucleic acids and total proteins.

The parallelism of the simian and human pancreatic changes in protein deficiency and kwashiorkor, respectively, tend to support the theory that the pancreas is the organ of primary injury in kwashiorkor.

The pancreatic lesions in protein deficiency in the monkey, and possibly kwashsiorkor, suggest a true cellular injury rather than a merely adaptive response.

Addendum

After acceptance of this paper, an article entitled "Effects of Postnatal Protein Deficiency on Weight Gain, Serum Proteins, Enzymes, Cholesterol, and Liver Ultrastructure in a Subhuman Primate (*Macaca mulatta*)" by J. M. Ordy, T. Samorajski, R. R. Zimmerman and P. M. Rady, was published in *The American Journal of Pathology*, May, 1966. The findings are essentially similar to those reported here in spite of the fact that those workers used younger monkeys.

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Initially, it was arranged by one of us (D. S.) to carry out these experiments utilizing material kindly put at our disposal by Professor V. Ramalingaswami in New Delhi. Unfortunately, the material prepared in New Delhi was poorly polymerized and could not be sectioned. Dr. M. G. Deo kindly visited our laboratory, however, and lent the benefit of his advice and experience in arranging these experiments.

We wish to acknowledge also the advice of Dr. E. Horilleno in carrying out laparotomies and liver biopsies, and the technical assistance of Mrs. Claire Nielsen, Mrs. Lynn Schmutz, Mrs. Faye Brady and Mrs. Dorothea Kuhar.

[*Illustrations follow*]

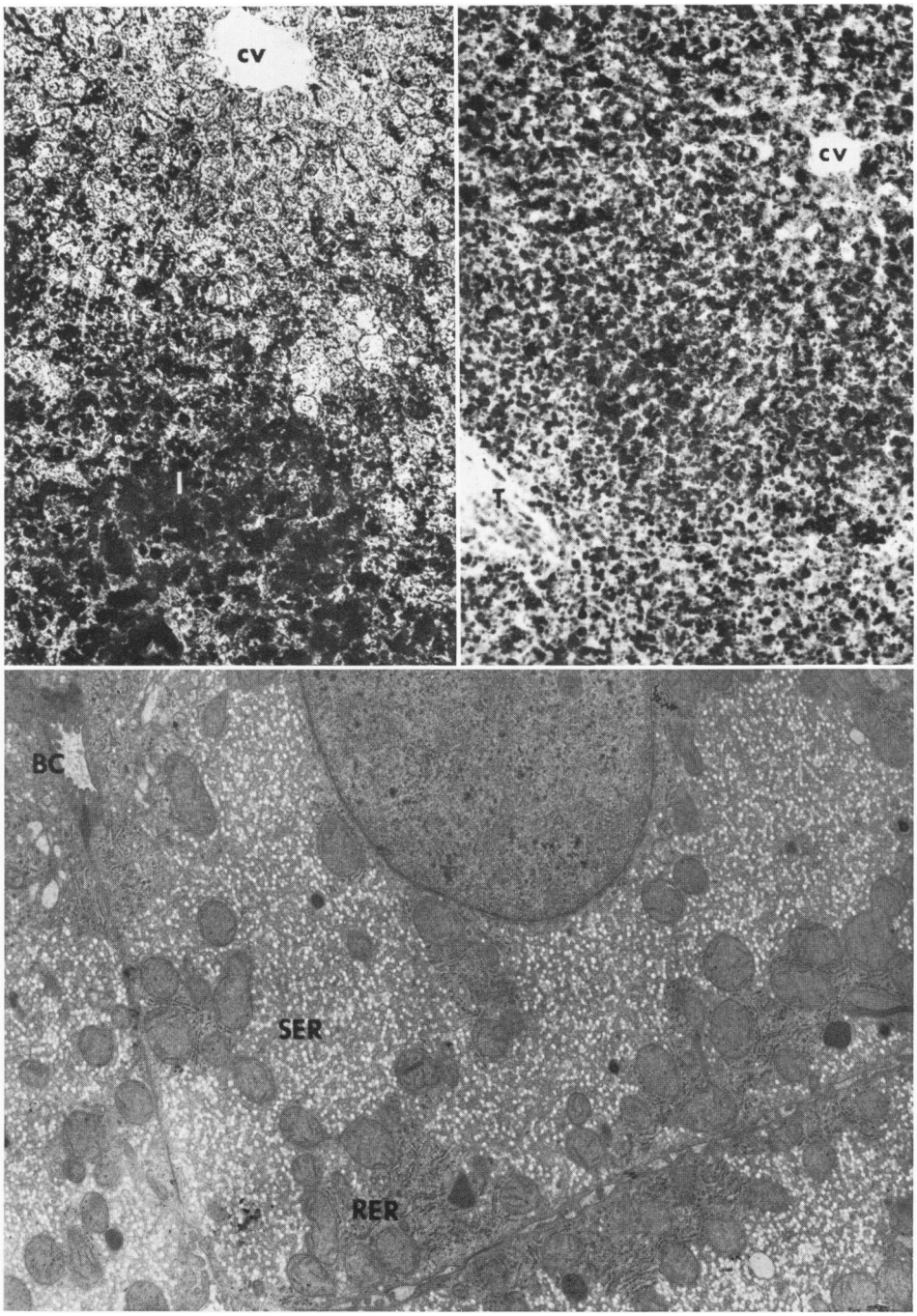
Legends for Figures

All electron micrographs are from sections double stained with lead and uranium.

Fig. 1. Liver. Protein deficient, monkey 6 (4 weeks). Lipid droplets (l) accumulate in the periportal area, with minimal or no fatty change in the centrilobular area. Central vein (cv). Oil red O stain. × 190.

Fig. 2. Liver. Protein deficient, monkey 7 (6 weeks). The periportal fatty change progresses to moderately advanced fat accumulation in the entire lobule, although biochemically the lipids were only twice the control level. Central vein (cv); portal triad (T). Oil red O stain. × 132.

Fig. 3. Liver. Control monkey 2. There is paucity of cytoplasmic organelles and the rough endoplasmic reticulum (RER) is not as prominent as in the rat. The smooth endoplasmic reticulum (SER) is hyperplastic and is intimately associated with glycogen (appearing as "holes"). Biliary canaliculus (BC). × 7,000.



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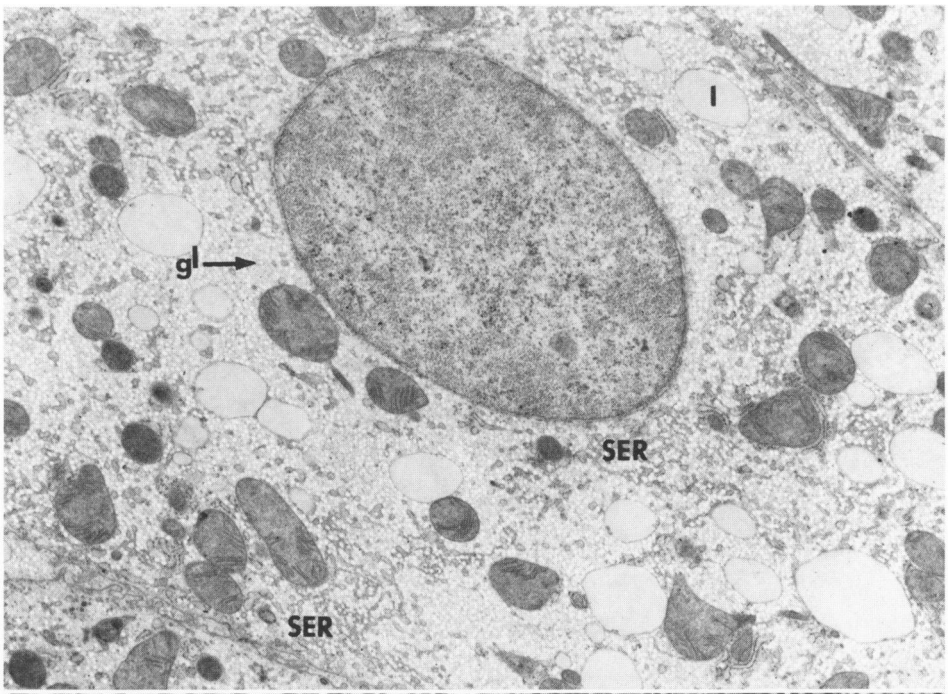

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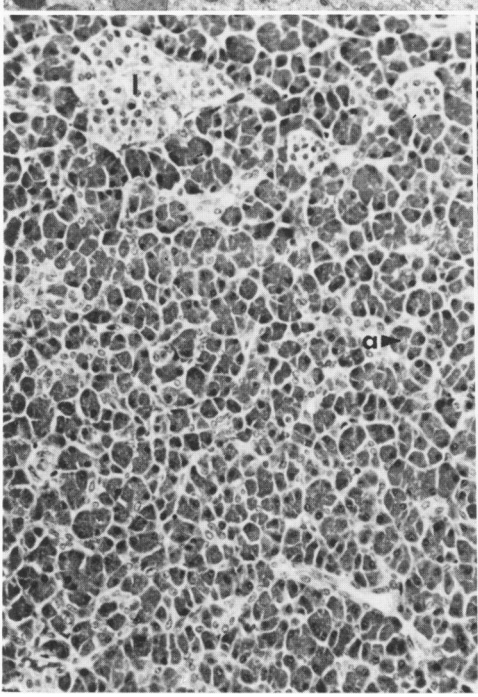

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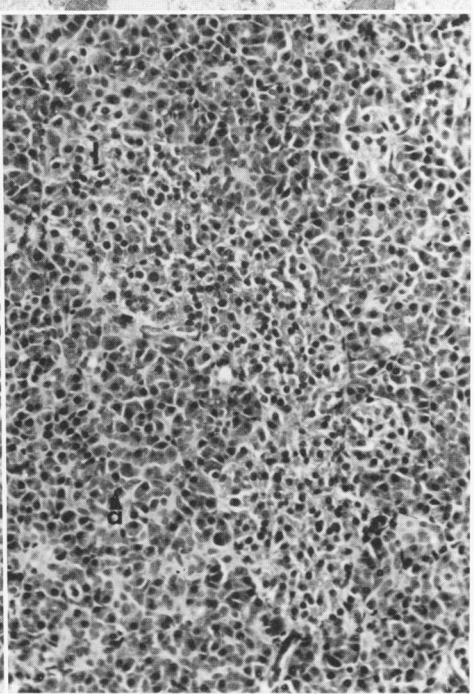

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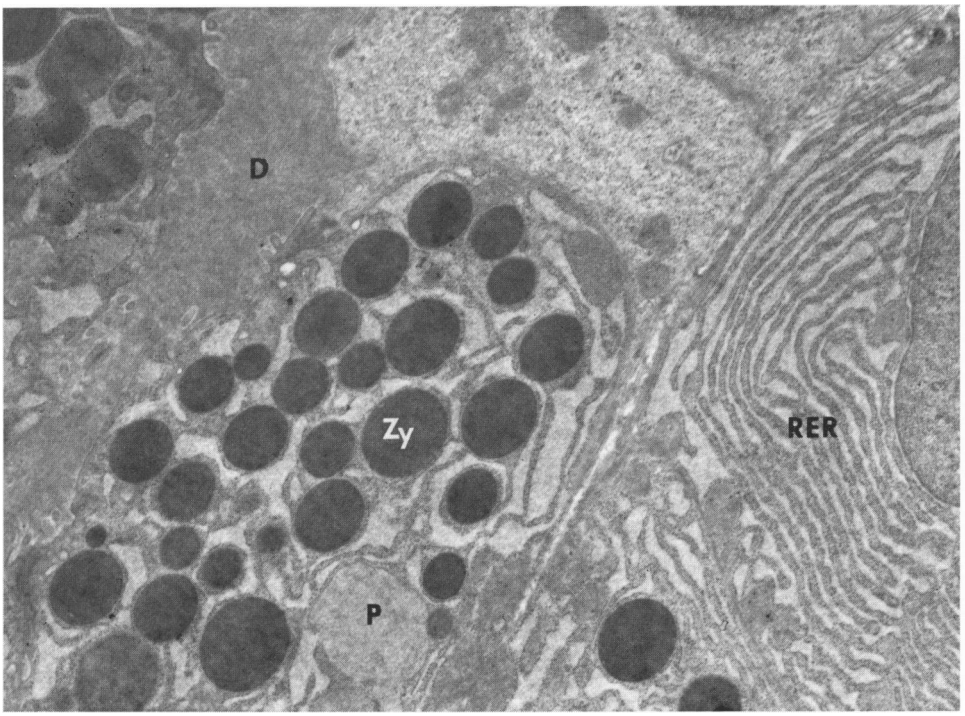

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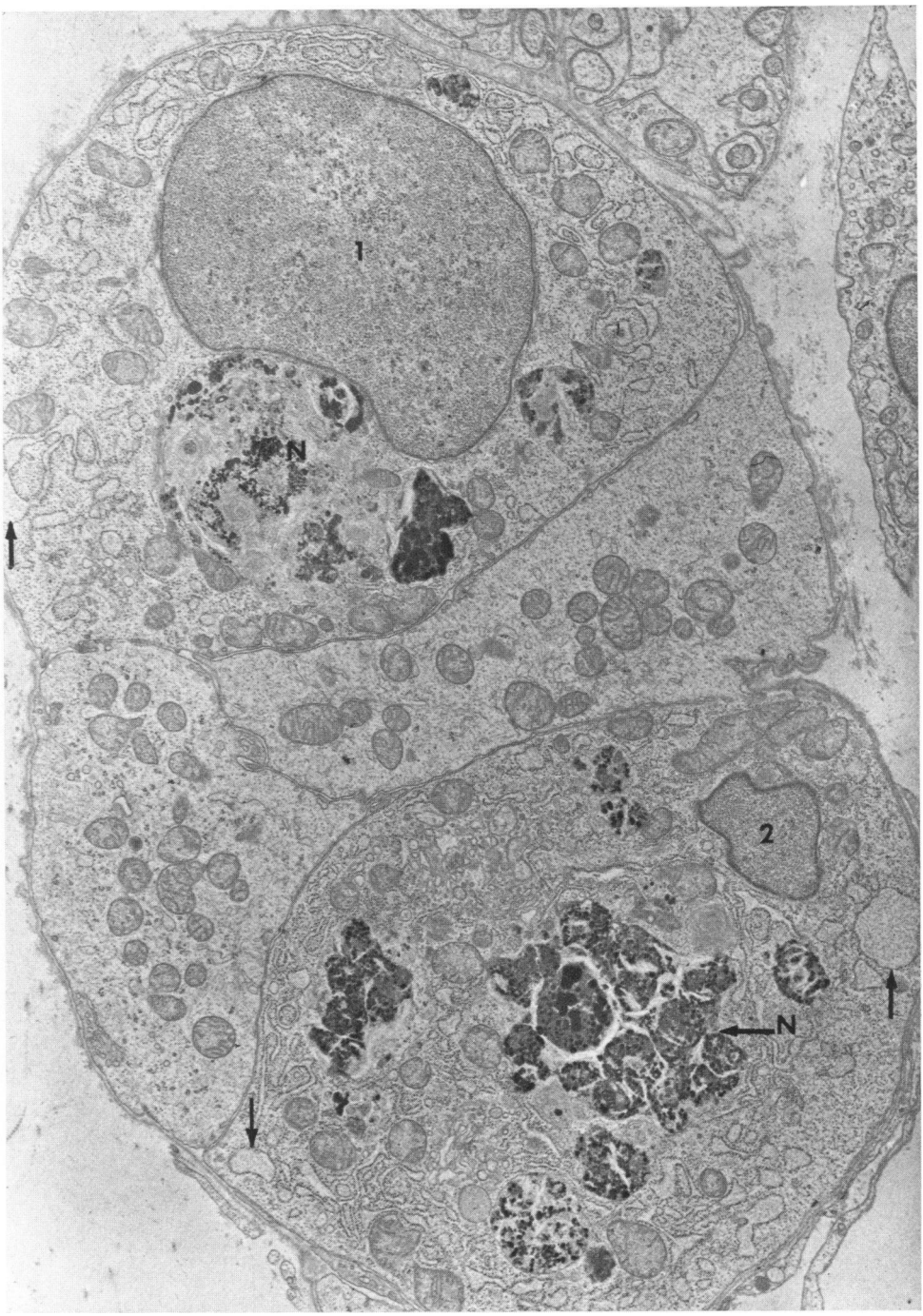
FIG. 4. Liver, protein deficient. Except for the accumulation of lipid bodies (l), the ultramicroscopic changes are slight. The SER is decreased, and is associated with glycogen particles, though large glycogen rich areas (glycogen, gl) are devoid of such membranes. × 5,800.

FIG. 5. Pancreas, normal, monkey 2. Acinar pattern (a) is evident and the acinar cells contain abundant zymogen granules. Islet (I). Gomori's trichrome stain. × 132.

FIG. 6. Pancreas, protein deficient. There is marked atrophy, loss of zymogen granules and apparent disorganization of the acinar pattern (a). Islet (I). Gomori's trichrome stain. × 132.

FIG. 7. Pancreatic acinar cell. Control monkey 2. A prominent component of the cell is the well developed RER with parallel profiles in the basal portion. Numerous zymogen (Zy) and few prozymogen (P) granules are seen in the apical portion of the cell. Acinar lumen. (D). × 9,600.



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Figures 8 to 12, and 14 are from the pancreas in monkeys on protein deficient diet for 6 to 8 weeks.

FIG. 8. The acinar cells (1 and 2) are atrophic. There is marked attenuation and disorganization of the RER, with focal dilatation (arrows). The attached ribosomes are also decreased. Focal areas of cytoplasmic necrosis (N) are numerous, and are represented by the highly osmiophilic areas. × 7,300.

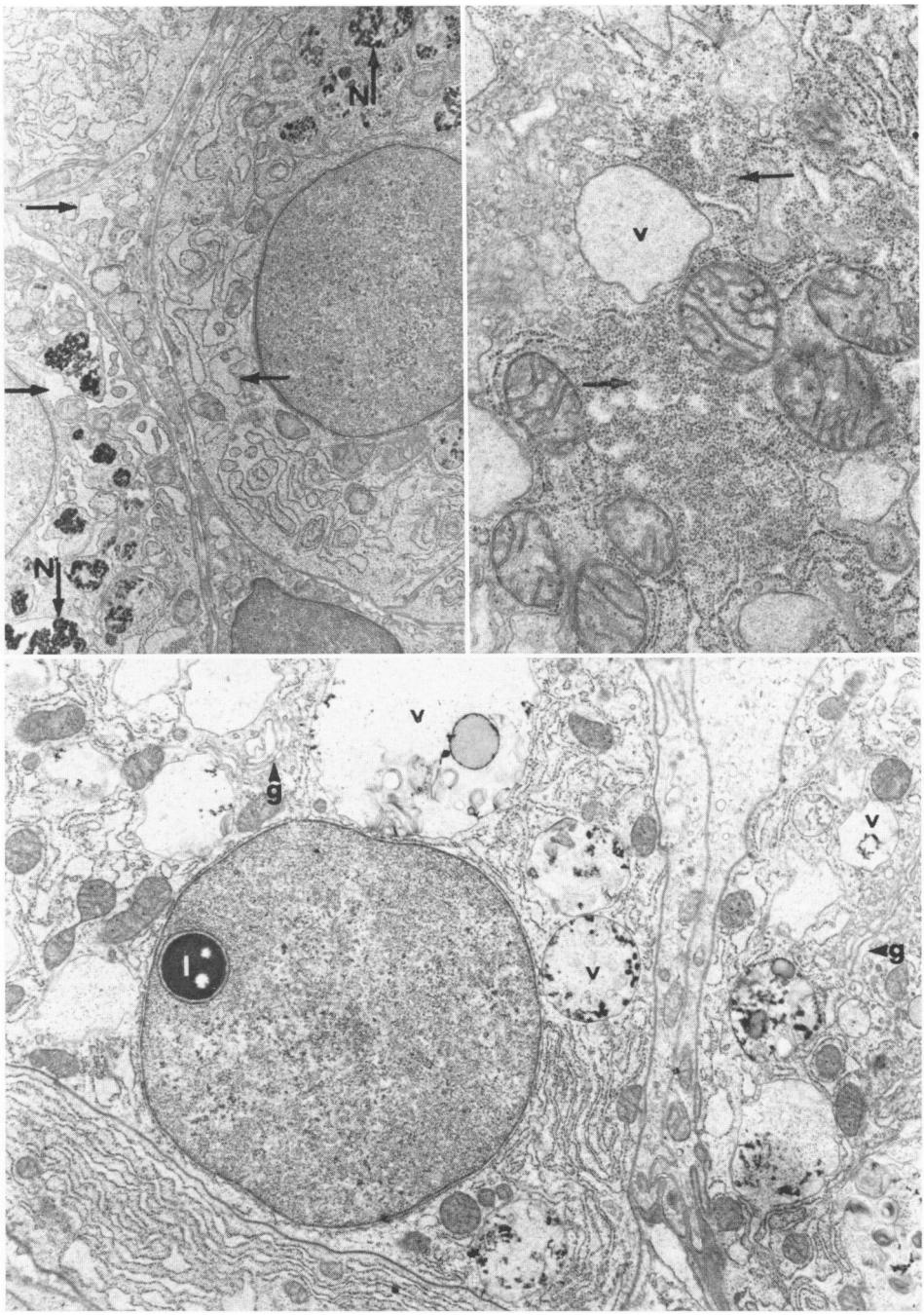


FIG. 9. Basal portions of 3 pancreatic acinar cells. The RER is irregularly attenuated, focally dilated (arrows), with relative increase in the intermembranous cytoplasmic matrix. Many areas of necrosis (N) appear in the cytoplasmic matrix. × 5,800.

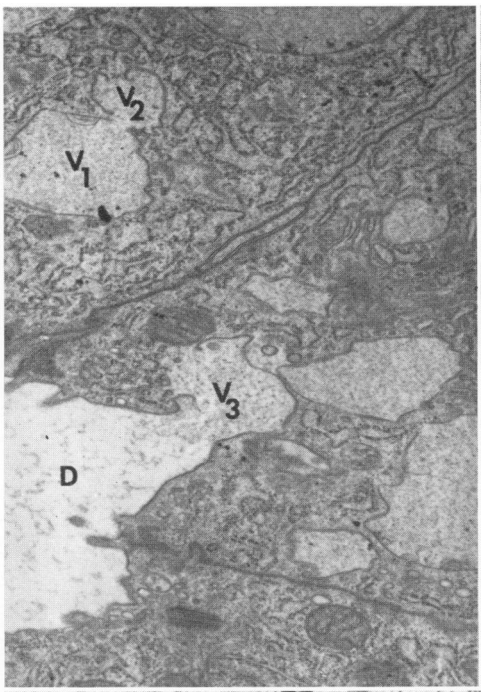
FIG. 10. Acinar cell. In some cells, the free polyribosomes (arrows) are increased in number and are often arranged in a spiral pattern. Golgi vacuole (v). × 14,400.

FIG. 11. Acinar cell. Vacuoles (v) of varying sizes are generally seen; these contain a fine granular material. Many vacuoles also contain irregular highly osmiophilic deposits or globules, and arrays of fibrillar material attached to their inner surface. Continuity with vacuoles of the Golgi complex (g) is often suggested by the transition of Golgi vacuoles and the larger degenerating vacuoles. Lipid in nucleus (l). × 7,500.

FIG. 12. Apical portion of acinar cell. Vacuoles (V), morphologically similar to those in Figure 11, are prominent. These are considered analogous to prozymogen granules because of the eventual secretion of their content into the lumen in a manner similar to the normal, e.g. fusion of vacuoles (V_1 and V_2), fusion of vacuole with the plasma membrane (V_3), and extrusion of contents into the lumen (D). The lumen content is similar to that of the vacuoles. × 11,700.

FIG. 13. Apical portion of acinar cell, normal monkey. The lumen content (D), in contrast to that seen in Figure 12, is highly dense and fibrillar. Zymogen granule (Zy); prozymogen granule (P). × 14,400.

FIG. 14. Acinar cell. A large area of cytoplasmic necrosis (N) is non-membrane limited. Concentric arrays of fibrillar material and highly osmiophilic deposits are seen along with recognizable portions of the cytoplasm. Rough endoplasmic reticulum (RER). × 11,700.



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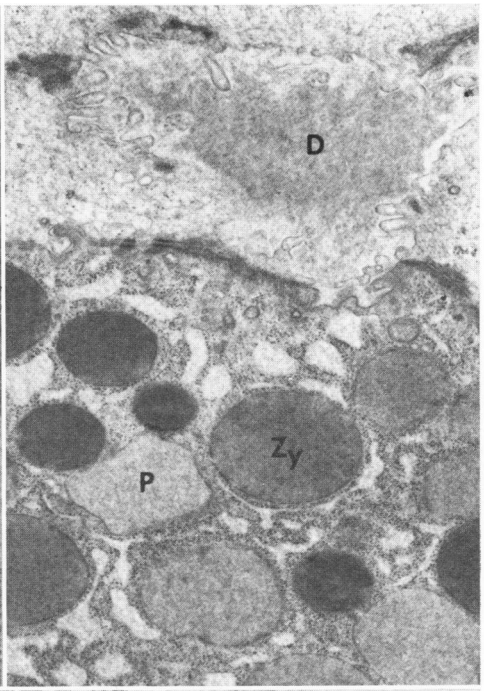

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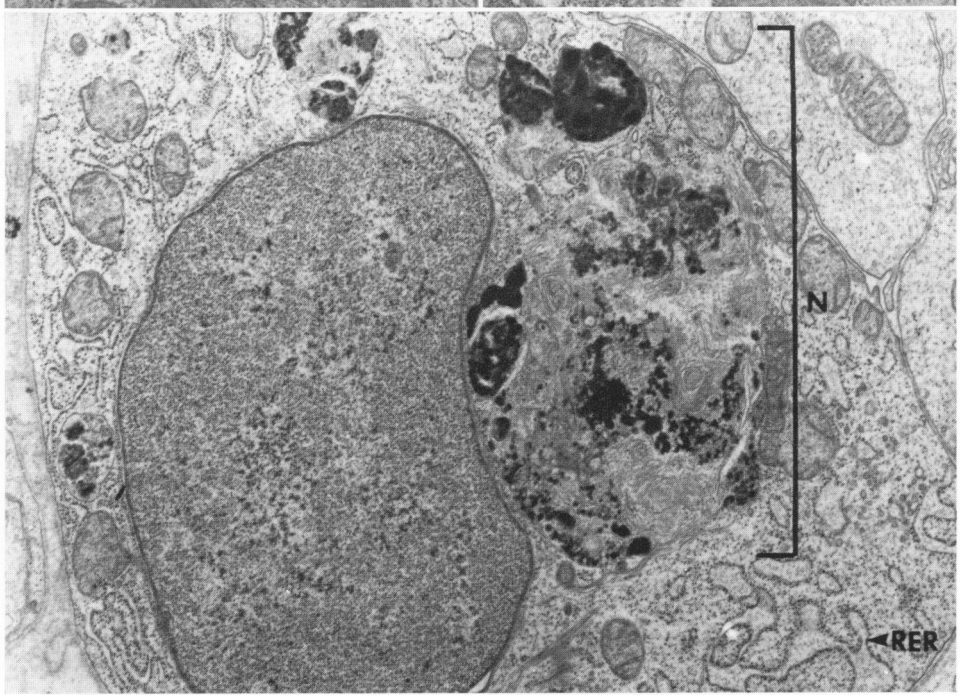

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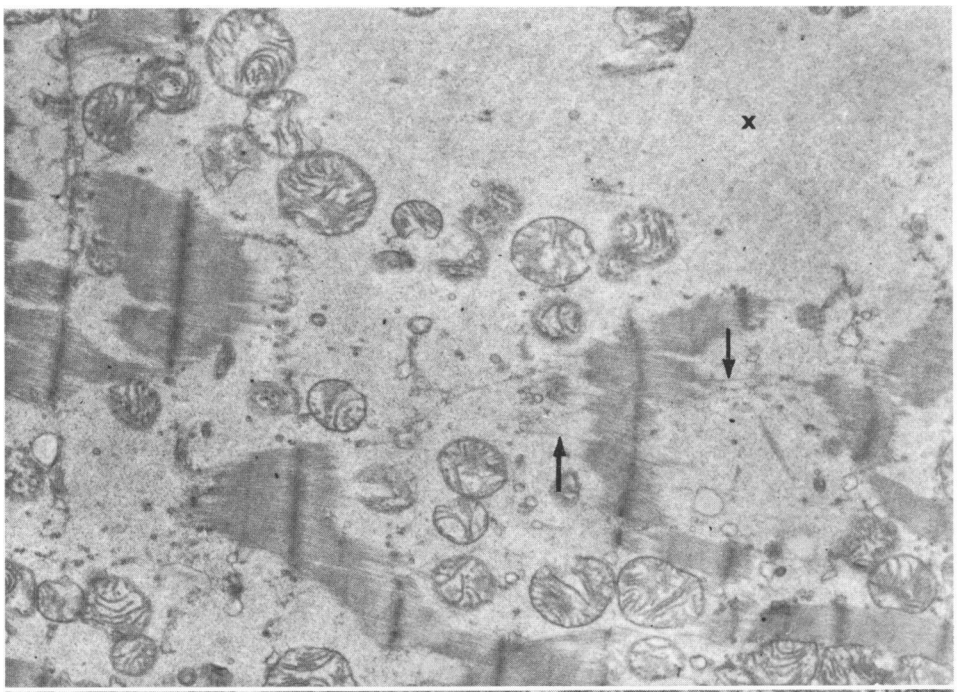
FIG. 15. Myocardium, protein deficient. Focal defects (X), devoid of myofibrils and containing few mitochondria, (some of which show a moth-eaten appearance) are apparent. In the margin of the defects, segmental loss of structure of one or more sarcomeres is seen and remnants of myofilaments (arrows) and membranes are still present. × 6,300.

FIG. 16. Striated muscle (sartorius), protein deficient. There are irregular segmental defects of myofibers, similar to those in the myocardium (Fig. 15). Destruction of the Z lines (*) is often seen. Enlarged mitochondria and remnants of myofilaments (arrows) appear in the defects. × 11,700.



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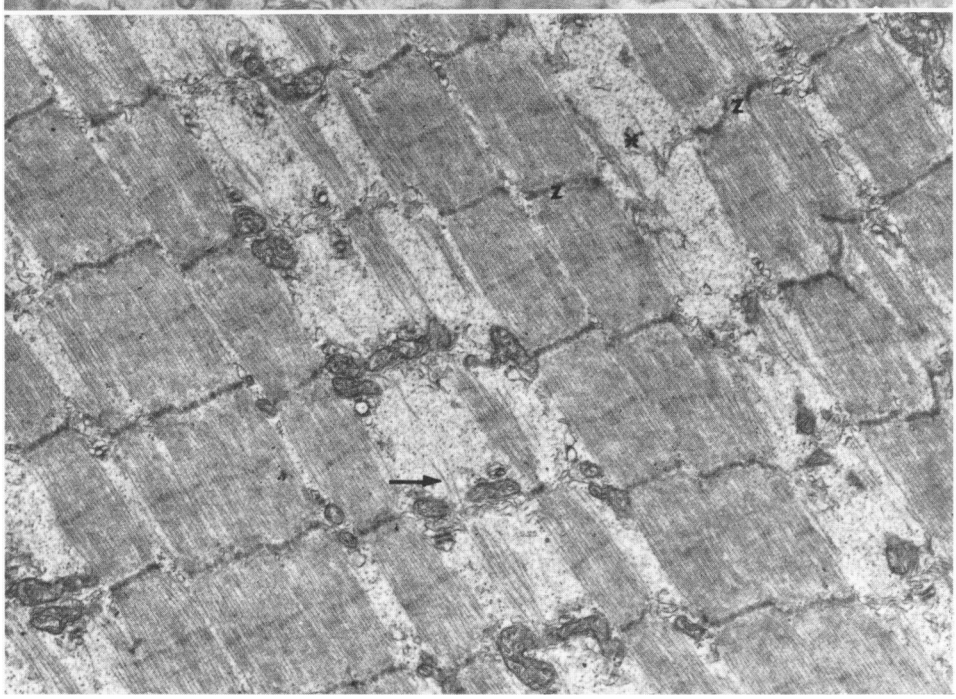

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