Virchows Arch. Abt. A Path. Anat. 360, 327—337 (1973)

Pancreatic and Hepatic Ultrastructure in Offspring of Rabbits with Protein Malnutrition

Klaus F. Wellmann, Masazumi Adachi, and Bruno W. Volk
Isaac Albert Research Institute of the Kingsbrook Jewish Medical Center, Brooklyn, N.Y.

Received May 18, 1973

Summary. Pregnant New Zealand rabbits were fed on a low-protein, low-fat, high-carbohydrate diet during the second half of gestation and during the subsequent period of lactation. Baby rabbits born to these protein-restricted does and suckled by them for two or more weeks showed loss of fur, stunted growth, fatty metamorphosis and increased glycogen storage in the liver, and atrophy of pancreatic acinar cells associated with loss of zymogen granules as well as widespread cytoplasmic vacuolization. It is suggested that the changes observed are not merely the effect of caloric restriction but also reflect a more specific nutritional deficiency (protein deficiency) transmitted by the mother rabbit to her offspring.

Introduction

A diet high in carbohydrate, low in fat and deficient in protein, simulating the diet of poor native Jamaicans, induces pancreatic acinar atrophy with reduction in the number of zymogen granules, a considerable decline in weight, loss of hair, and fatty metamorphosis of the liver often terminating in cirrhosis as was demonstrated by light and electron microscopy as well as in histochemical studies on adult rabbits (Volk and Lazarus, 1960; Lazarus and Volk, 1964, 1965). Since it is well known that malnutrition during gestation may adversely affect the development of the fetus, it was decided to investigate the ultrastructural alterations, if any, in the pancreases and livers of neonatal and young suckling rabbits whose mothers had been subjected to the same protein deficient diet during the second half of their pregnancies and during the subsequent periods of lactation.

Material and Methods

A total of 31 neonatal and suckling white New Zealand rabbits, the offspring of ten does, were utilized. Six of the mother rabbits had received a high carbohydrate, low fat and low protein diet during the last two weeks of gestation as well as during the entire period of lactation. Composition and preparation of the deficient diet have been described elsewhere (Volk and Lazarus, 1960). In short, it consisted of white potatoes (20%), green bananas (22.5%), corn meal (30%), brown sugar (20%), and powdered whole milk (7.5%); the calculated content of protein was 5.8%; fat, 2,2%; and carbohydrate, 73.9%. Twenty-two baby rabbits born to these six mothers were sacrificed, by overdoses of sodium Nembutal, at intervals ranging from three days to seven weeks after their delivery. The six mother rabbits were weighed once weekly and were killed after they had ingested the deficient diet for a period of three months each.

Supported by a grant from the National Institutes of Health (A-2203).
Presented at the 70th Annual Meeting of the American Association of Pathologists and Bacteriologists, Washington, D. C., February 26, 1973.

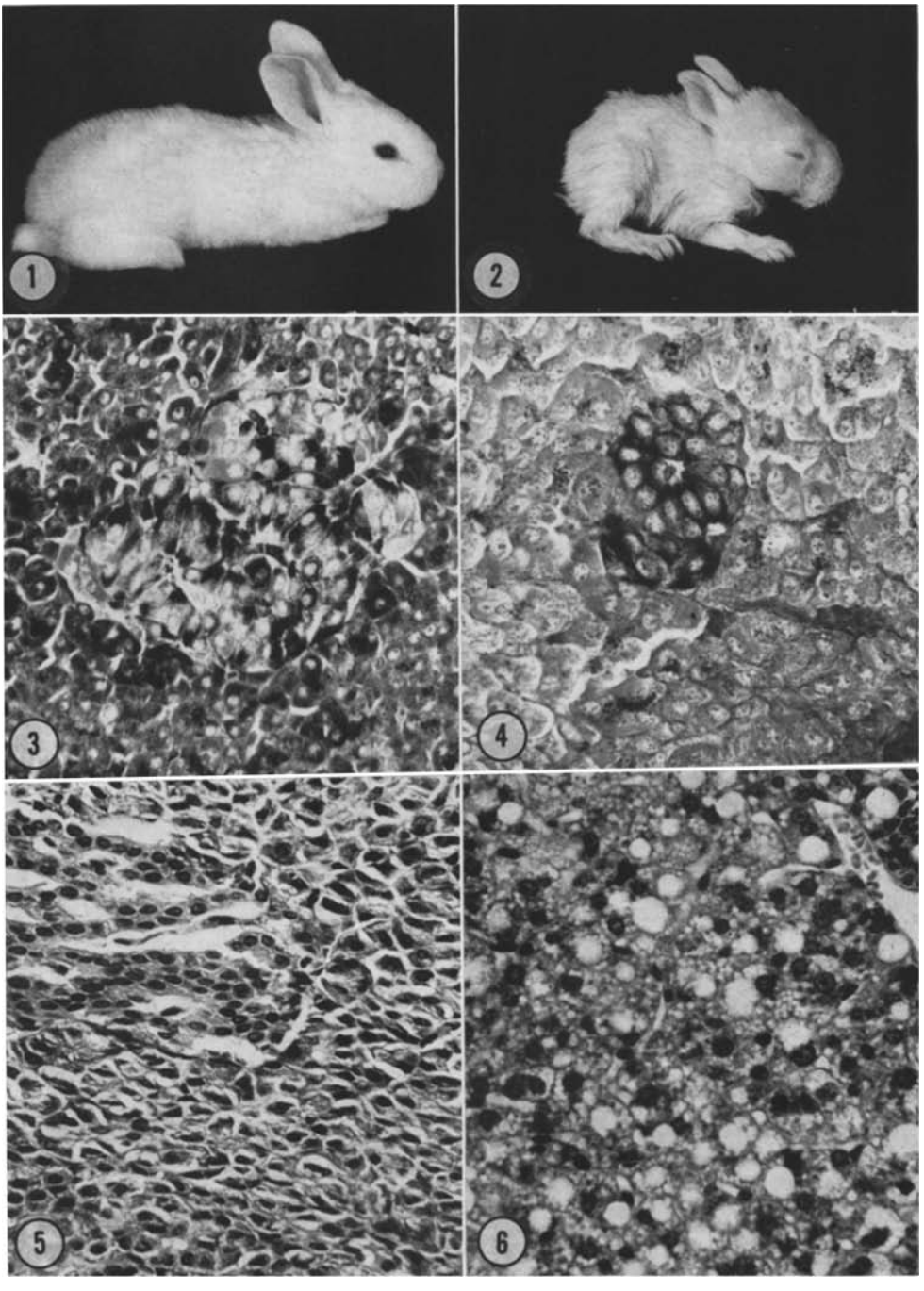


Fig. 1. 19 day old rabbit (mother on normal diet). × 1/2

Fig. 2. 19 day old rabbit (mother on deficient diet). Stunted growth, ruffled hair and abnormal posture are evident. × 1/2

The remaining four does were fed regular Purina rabbit chow. Nine baby rabbits born to them were sacrificed at intervals from two days to seven weeks and served as normal controls.

All does and their respective offspring were housed in separate metabolic cages and received tap water ad libitum.

Portions of liver and pancreas were fixed in formalin, and paraffin-embedded sections were stained with hematoxylin and eosin. Some pancreatic tissue was fixed in Zenker-formol solution, and sections were stained with a modified aldehyde-fuchsin-trichrome method as previously described (Lazarus and Volk, 1962).

For ultrastructural studies, diced blocks, measuring less than 1 mm in greatest diameter, of pancreas and liver were fixed for two hours in ice-cold 1 per cent osmic acid solution containing 20.83 per cent acetate-Veronal buffer, as well as 4.5 per cent sucrose (Caulfield, 1957), adjusted to a pH of 7.4 and embedded with epoxy resin (Luft, 1961). Ultrathin sections were then cut with glass knives on a microtome, stained with lead citrate and uranyl acetate (Reynolds, 1963), and viewed and photographed with an electron microscope.

Results

Gross Changes

All rabbits born to protein-deficient does displayed stunted growth, ruffled fur, reduced motor activity, and abnormal posture when compared with animals of the same age whose mothers had been fed a normal diet (Figs. 1 and 2). These changes varied in intensity between litters but had generally become quite obvious by the end of the second week of life. They progressed in severity as the animals matured.

There was no significant weight loss in the mother rabbits before they gave birth to their young, approximately two weeks after they had been placed on the protein-restricted diet. During the following three months a gradual reduction in body weight took place in these does which attained 20 to 35 per cent of their initial levels.

Light Microscopy

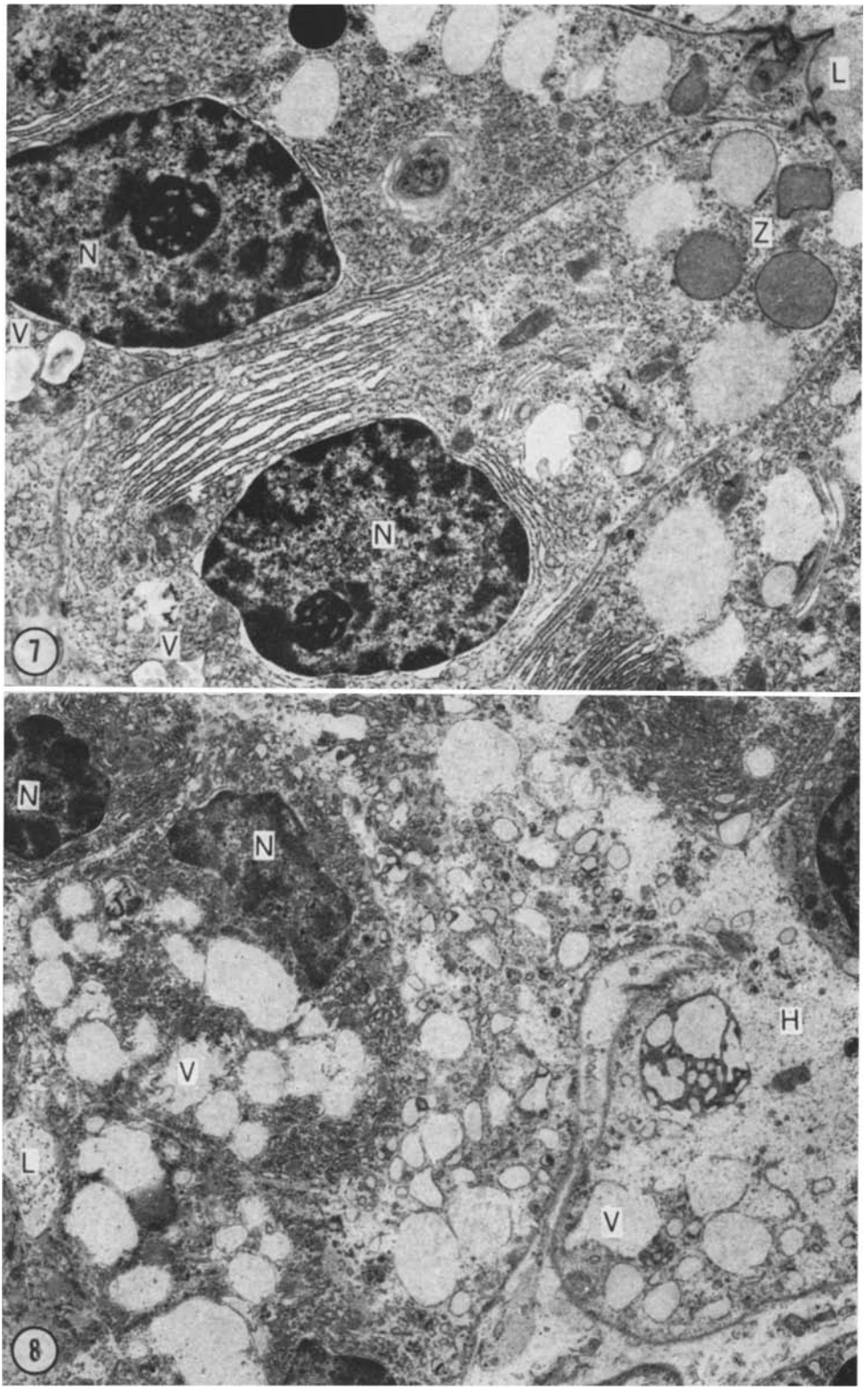
Pancreas. In baby rabbits born to normally fed does, both the islet cells and the acinar cells of the pancreas appeared well granulated in aldehyde-fuchsin-trichrome-stained sections (Fig. 3). In contrast, poor acinar (but not islet) cell granulation was often observed in rabbits born to protein-deprived mothers,

Fig. 3. Light micrograph of pancreas of 41 day old rabbit (mother on normal diet). Islet and exocrine cells are well granulated. Aldehydefuchsin-trichrome stain. × 375

Fig. 4. Pancreas of 34 day old rabbit (mother of deficient diet). Whereas the islet cells appear well granulated, only a few stainable zymogen granules are visible in the exocrine acinar cells. Aldehydefuchsin-trichrome stain. × 500

Fig. 5. Pancreas of 47 day old rabbit (mother on deficient diet). The exocrine acinar cells appear atrophic, lack cohesion with one another, and frequently contain dark, pyknotic nuclei, as well as small cytoplasmic vacuoles. Hematoxylin and eosin stain. × 375

Fig. 6. Periportal region of liver of 15 day old rabbit (mother on deficient diet). Small and large fat vacuoles are present in virtually all hepatocytes of this field. Hematoxylin and eosin stain. × 375



Figs. 7 and 8

especially after the second week of life (Fig. 4). During the later stages of the experiment, the exocrine acinar cells of many of these animals were distinctly atrophic and lacked cohesion with one another; their nuclei had become dark and pyknotic and fine cytoplasmic vacuoles were sometimes seen (Fig. 5).

Liver. Small and large lipid vacuoles were noted in the hepatocytes of several but not all baby rabbits born to protein-deficient does, as early as at the end of the second week of their lives (Fig. 6). Fibrosis or cirrhosis were absent, however. No lesions of any kind were present in the livers of the control animals.

Electron Microscopy

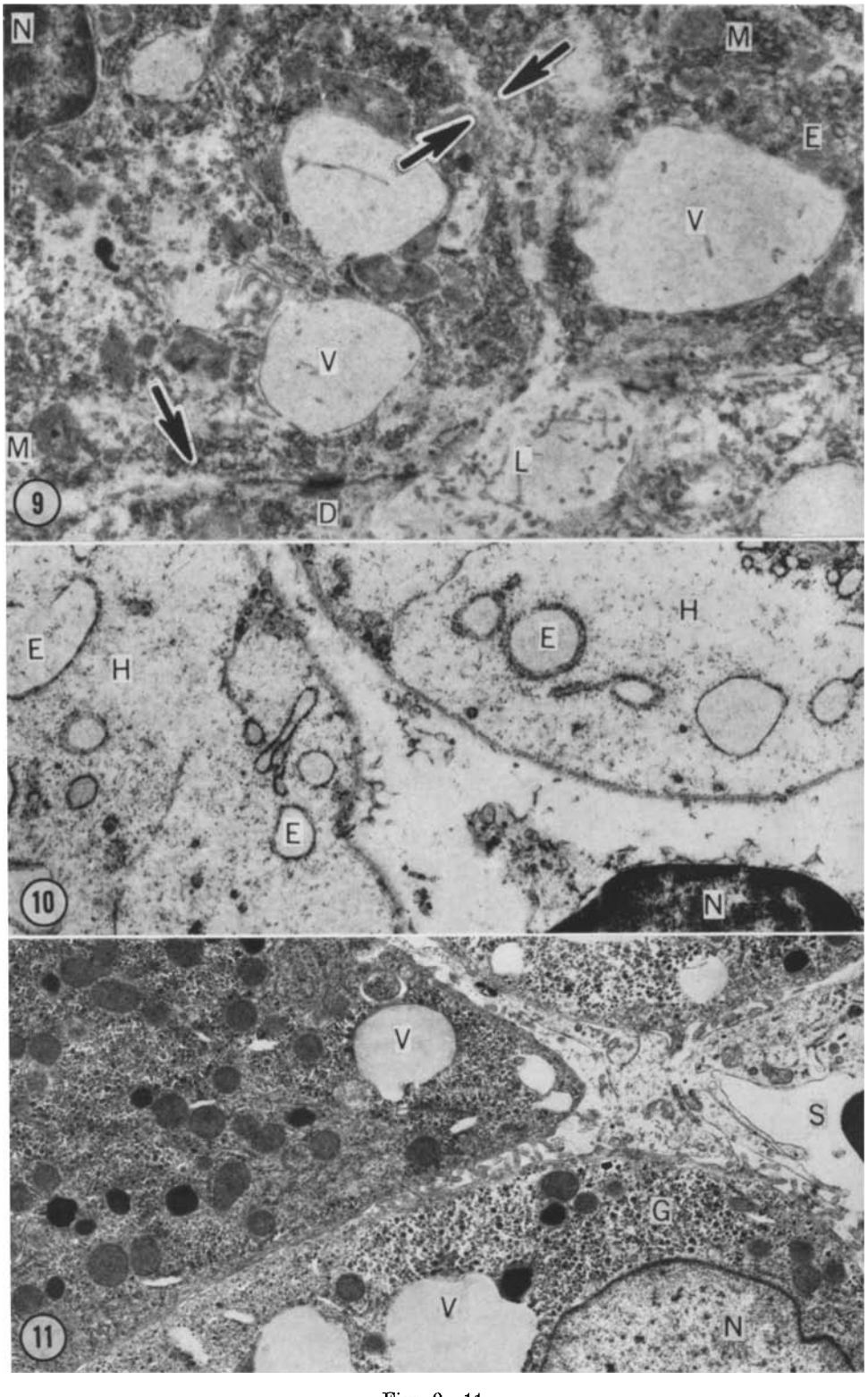
Pancreas. In baby rabbits born to protein-restricted does and sacrificed within two weeks after delivery, no definitely abnormal ultrastructural alterations were demonstrable except for occasional small membrane-enclosed vacuoles, with or without amorphous debris, in the basal portions of some exocrine acinar cells (Fig. 7). Cellular organization and organelles appeared intact, and zymogen granule formation proceeded normally. In many rabbits more than two weeks old, however, distinctly pathological changes became evident. Most conspicuous was the appearance of increasingly larger numbers of membrane-enclosed cytoplasmic vacuoles which were empty or contained some electron-lucent, amorphous substance (Figs. 8 and 9). The nuclei of the affected exocrine acinar cells displayed convoluted contours and clumping of chromatin (Figs. 8 and 9). Portions of the hyaloplasm appeared largely devoid of structured components (Figs. 9 and 10), and the units of the endoplasmic reticulum showed vesicular distension (Fig. 10). Cohesion between neighboring acinar cells was frequently poor or lacking (Fig. 9), and zymogen granule formation was severely suppressed (Figs. 8 and 9). There were no significant ultrastructural alterations in the cells of the islets of Langerhans of these baby rabbits.

The pancreases of all does that had received the protein-deficient diet for three months displayed light and electron microscopic changes similar to those described above and identical to the ones reported earlier in nonpregnant adult rabbits (Lazarus and Volk, 1964, 1965). These included, in particular, loss of zymogen granules, acinar cell atrophy, and cytoplasmic vacuolization. No such alterations were noted in the pancreases of the control animals, either does or baby rabbits.

Liver. Occasional, relatively small membrane-enclosed vacuoles filled with electron-lucent amorphous contents were seen in infants of normally fed control rabbits (Fig. 11). As early as five days after their birth (Fig. 12), but especially after several weeks (Figs. 13 and 14), the hepatocytes of the

Fig. 7. Pancreatic acinar cells of 3 day old rabbit (mother on deficient diet). Cellular organization and organelles appear intact. A few zymogen granules (*Z*) are present. The basal portions contain small membrane-enclosed vacuoles (*V*). *L* Centroacinar lumen; *N* nuclei. × 7500

Fig. 8. Pancreas of 21 day old rabbit (mother on deficient diet). The atrophic acinar cells contain dense, convoluted nuclei (*N*). There are numerous cytoplasmic membrane-enclosed vacuoles (*V*) and edematous portions (*H*). Zymogen granules are lacking. *L* Centroacinar lumen. × 4400



Figs. 9—11

majority of baby rabbits born to does on the deficient diet contained similar but much larger membrane-clad vacuoles which, in the older rabbits, took up most of the cytoplasm (Fig. 14). Collections of dense bodies, composed of concentrically arranged lamellae, were sometimes seen (Fig. 12). Distinctly increased amounts of glycogen were noted in a large number of hepatocytes (Figs. 13 and 14).

In the hepatocytes of the mother rabbits sacrificed after they had received the deficient diet for three months, ultrastructural alterations similar to those in their offspring were observed.

Discussion

It has been established that maternal malnutrition during mammalian gestation profoundly affects the offspring. A balanced but calorically deficient diet given to the pregnant mother induces significant growth retardation of body and organs of the fetus (Chow and Lee, 1964; Naeye, 1971). If calorically adequate but protein-deficient diets are fed to gestating and lactating animals, litter sizes and weights of the individual fetuses will decrease, and many neonates will not survive to weaning age (Seegers, 1937; McCoy, 1940; Curtiss, 1953; Macomber, 1953; Nelson and Evans, 1953; Leathem, 1959; Zeman, 1967). Lactation failure in the dam, coupled with the retarded neonates' impaired ability to suckle, was the apparent cause of death in pups nursed by protein-restricted rats (Zeman, 1967). The milk of such dams was found reduced in quantity but had maintained its usual protein concentration (Venkatachalam and Ramanathan, 1964).

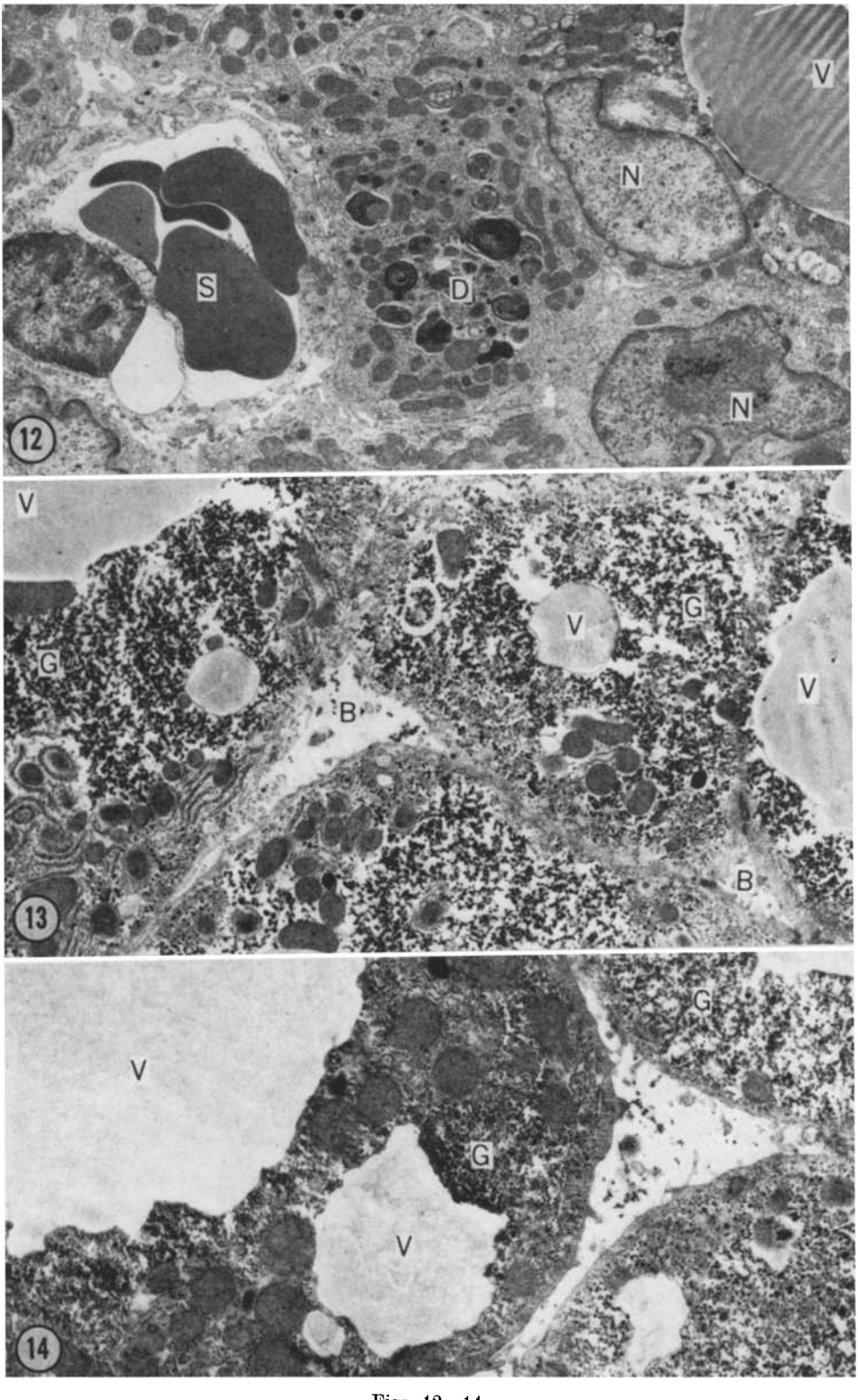
The critical need for protein is highest during the earliest stages of placental and fetal development. In other words, the later during gestation protein intake is being restricted, the better will be both reproductive success and survival of neonates (Nelson and Evans, 1953). In the present experiment, the protein-deficient diet was initiated only at mid-term during pregnancy, and, consequently, survival of offspring was excellent.

In general, morphological alterations in the pancreases and livers of baby rabbits were first noted after their mothers had been subjected to deficient protein intake for about four weeks (two weeks during pregnancy and two during lactation). These changes were quite apparent before the young rabbits began to ingest solid foods on their own which, in this species, generally does not occur before the beginning of the fourth postnatal week (Manual, 1961). Qualitatively, the observed alterations were identical with those described earlier in adult rabbits

Fig. 9. Same animal as in Fig. 8. A desmosome (*D*) is present, but intercellular cohesion is lacking elsewhere (arrows). There are cytoplasmic vacuoles (*V*) but no zymogen granules. The mitochondria (*M*) appear condensed. *E* Endoplasmic reticulum; *L* centroacinar lumen. × 13875

Fig. 10. Basal portions of exocrine pancreatic acinar cells of 22 day old rabbit (mother on deficient diet). The hyaloplasm (*H*) appears edematous, and the units of the endoplasmic reticulum (*E*) form vesicles. *N* Nucleus. × 10500

Fig. 11. Liver of 21 day old control rabbit (mother on normal diet). A few, relatively small cytoplasmic vacuoles (*V*) are visible. *G* Glycogen granules; *N* nuclei; *S* sinusoidal space. × 7500



Figs. 12—14

fed the same protein-deficient diet for several weeks (Volk and Lazarus, 1960; Lazarus and Volk, 1964, 1965). They were also similar to the pancreatic and hepatic lesions induced by protein restriction in other species (Weisblum *et al.*, 1962; Sidransky and Verney, 1964; Svoboda *et al.*, 1964, 1966; Deo *et al.*, 1965; Enwonwu and Sreebny, 1970) as well as to some of the morphologic alterations seen in human patients with kwashiorkor (Trowell *et al.*, 1954; Camain *et al.*, 1959; Theron and Liebenberg, 1963; Blackburn and Vinijchaikul, 1969).

While loss of fur, reduced motor activity, extensive hepatic glycogen deposits, fatty metamorphosis of the liver, and widespread pancreatic acinar cell vacuolization—all present in the baby rabbits of this experiment—are characteristic sequelae of chronic protein deficiency, such lesions are not generally found in rabbits fed a calorically insufficient but balanced diet even though the total weight loss in both groups may be the same (Lazarus and Volk, 1965). This would suggest that the morphologic changes observed in the young of protein-restricted does are not merely the effect of partial starvation but reflect a more specific nutritional deficiency transmitted from the mother rabbit to her offspring.

The striking atrophy of pancreatic acinar cells in protein malnutrition is very likely related to the high rate of amino acid utilization for enzyme synthesis by this organ. It has been shown that of all organs the pancreas forms the greatest amount of protein per unit weight (Friedberg *et al.*, 1948). Therefore, a deficiency of protein or of amino acids is apt to quickly reduce available pancreatic enzyme protein precursors. Protein inhibitors, such as the phenylalanine analogue B-3-thienylalanine, cause similar morphologic changes including a suppression of zymogen granule formation and acinar cell vacuolization (Hruban *et al.*, 1962) whereas methionine deficiency induced by ethionine administration is followed by somewhat different pancreatic alterations (Farber and Popper, 1950; Herman and Fitzgerald, 1962).

In contrast to the considerable reduction of the number of zymogen granules in many exocrine acinar cells of the baby rabbits on the restricted diet, the B cells of their pancreatic islets remained well granulated throughout the experiment. Even though large amounts of cysteine are required for insulin synthesis, and rabbits on a diet low in methionine and cysteine have been found to display a decreased pancreatic insulin content (Griffith, 1950), it was only after very prolonged exposure of adult rabbits to the protein-deficient diet that some visible loss of B cell granules did occur (Volk and Lazarus, 1960). These observations may perhaps be related to the fact that the enzyme systems of endo- and exo-

Fig. 12. Liver of 5 day old rabbit (mother on deficient diet). A large membrane-enclosed cytoplasmic vacuole (*V*) and a cluster of dense bodies (*D*) are noted. *N* Nuclei; *S* sinusoidal space. × 7500

Fig. 13. Liver of 28 day old rabbit (mother on deficient diet). Large cytoplasmic vacuoles (*V*) and dense glycogen deposits (*G*) are visible. *B* Bile canaliculi. × 5800

Fig. 14. Liver of 42 day old rabbit (mother on deficient diet). The cytoplasm of the hepatocytes contains much glycogen (*G*) and is compressed into peripheral crescents by huge, membrane-enclosed vacuoles only portions of which are illustrated (*V*). × 7500

crine pancreatic cells react differently to protein restriction. Thus, acid phosphatase activity all but ceases at the secretory pole of exocrine acinar cells in rabbits on protein-poor diets whereas islet cell acid phosphatase remains unaffected (Lazarus and Volk, 1964, 1965).

In human kwashiorkor it has been surmised that the pancreatic lesions precede the hepatic alterations (Davies, 1948) and that the latter depend on the loss of pancreatic enzymes from the intestine which is demonstrable in this disease (Davies, 1948; Gillman and Gillman, 1951; Theron and Liebenberg, 1963). However, in the present study on baby rabbits, as well as in previously recorded observations on adult animals (Volk and Lazarus, 1960), lipid globules appeared in the hepatocytes at the same time at which pancreatic acinar cell alterations became evident; occasionally, in fact, they manifested themselves considerably earlier (Fig. 12). This suggests that the hepatic lesions in protein malnutrition are a direct result of the deficient diet and are not secondary to the pancreatic changes.

References

Blackburn, W. R., Vinijchaikul, K.: The pancreas in kwashiorkor: An electron microscopic study. Lab. Invest. **20**, 305–318 (1969)

Camain, R., Rouiller, C., Dupin, H.: Evolution de la stéatose hépatique dans le kwashiorkor sous l'influence du régime hyperprotidique. Étude en microscopie normal et électronique. Ann. Anat. path. **4**, 220–241 (1959)

Caulfield, J. B.: Effects of varying the vehicle for OsO_4 in tissue fixation. J. biophys. biochem. Cytol. **3**, 827–830 (1957)

Chow, B. F., Lee, C. J.: Effect of dietary restriction of pregnant rats on body weight gain of offspring. J. Nutr. **82**, 10–18 (1964)

Curtiss, C.: Effects of a low protein intake on the pregnant rat. Metabolism **2**, 344–353 (1953)

Davies, J. N. P.: The essential pathology of kwashiorkor. Lancet **1948II**, 317–320

Deo, M. G., Sood, S. K., Ramalingaswami, V.: Experimental protein deficiency: Pathological features in the rhesus monkey. Arch. Path. **80**, 14–23 (1965)

Enwonwu, C. O., Sreebny, L. M.: Experimental protein-calorie malnutrition in rats: Biochemical and ultrastructural studies. Exp. molec. Path. **12**, 332–353 (1970)

Farber, E., Popper, H.: Production of acute pancreatitis with ethionine and its prevention by methionine. Proc. Soc. exp. Biol. (N.Y.) **74**, 838–840 (1950)

Friedberg, F., Tarver, H., Greenberg, D. M.: The distribution pattern of sulfur-labeled methionine in the protein and the free amino acid fraction of tissues after intravenous administration. J. biol. Chem. **173**, 355–361 (1948)

Gillman, J., Gillman, T.: Perspectives in human malnutrition: A contribution to the biology and disease from a clinical and pathological study of chronic malnutrition and pellagra in the African, p. 201–213. New York: Grune & Stratton 1951

Griffiths, M.: The mechanism of the diabetogenic action of uric acid. J. biol. Chem. **184**, 289–298 (1950)

Herman, L., Fitzgerald, P. J.: The degenerative changes in pancreatic acinar cells caused by DL-ethionine. J. Cell Biol. **12**, 277–296 (1962)

Hruban, Z., Swift, H., Wissler, R. W.: Effect of B-3-thienylalanine on the formation of zymogen granules of exocrine pancreas. J. Ultrastruct. Res. **7**, 359–372 (1962)

Lazarus, S. S., Volk, B. W.: The pancreas in human and experimental diabetes, p. 257–267. New York: Grune & Stratton, 1962

Lazarus, S. S., Volk, B. W.: Electron microscopy and histochemistry of rabbit pancreas in protein malnutrition (experimental kwashiorkor). Amer. J. Path. **44**, 95–111 (1964)

Lazarus, S. S., Volk, B. W.: Ultrastructure and acid phosphatase distribution in the pancreas of rabbits: A comparison of alterations following protein deficiency and semistarvation diets. Arch. Path. **80**, 135–147 (1965)

Leathem, J. H.: Extra-gonadal factors in reproduction. In: Lloyd, C. W., ed. Recent progress in the endocrinology of reproduction, p. 179–203. New York: Academic Press, 1959

Luft, J. H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409–414 (1961)

Macomber, D.: Studies of reproduction in the rat. I. The effect of changes in the protein upon the fertility, pregnancy and lactation. New Engl. J. Med. **209**, 1105–1109 (1953)

Manual for Laboratory Animal Care, Section 2, p. 21, Ralston Purina Company, St. Louis, Missouri (1961)

McCoy, R. H.: Effects of different levels of protein in the diet of the rat. J. biol. Chem. **133**, 64–65, Abstract (1940)

Naeye, R. L., Diener, M. M., Harcke, H. T., Jr., Blanc, W. A.: Relation of poverty and race to birth weight and organ and cell structure in the newborn. Pediat. Res. **5**, 17–22 (1971)

Nelson, M. M., Evans, H. M.: Relation of dietary protein levels to reproduction in the rat. J. Nutr. **51**, 71–84 (1953)

Reynolds, E. S.: The use of lead citrate at high pH as an electron-opaque stain for electron microscopy. J. Cell Biol. **17**, 208–212 (1963)

Seegers, W. H.: The effect of protein deficiency on the course of pregnancy. Amer. J. Physiol. **119**, 474–479 (1937)

Sidransky, H., Verney, E.: Chemical pathology of acute amino acid deficiencies. VII. Morphologic and biochemical changes in young rats force-fed arginine-, leucine, isoleucine-, or phenylalanine-devoid diets. Arch. Path. **78**, 134–148 (1964)

Svoboda, D., Grady, H., Higginson, J.: The effects of chronic protein deficiency in rats. II. Biochemical and ultrastructural changes. Lab. Invest. **15**, 731–749 (1966)

Svoboda, D., Higginson, J.: Ultrastructural changes produced by protein and related deficiencies in the rat liver. Amer. J. Path. **45**, 353–379 (1964)

Theron, J. J., Liebenberg, N.: Some observations on the fine cytology of the parenchymal liver cells in kwashiorkor patients. J. Path. Bact. **86**, 109–112 (1963)

Trowell, H. C., Davies, J. N. P., Dean, R. F. A.: Kwashiorkor. London: Edward Arnold, Ltd. 1954

Venkatachalam, P. S., Ramanathan, K. S.: Effect of protein deficiency during gestation and lactation on body weight and composition of offspring. J. Nutr. **84**, 38–42 (1964)

Volk, B. W., Lazarus, S. S.: Rabbit pancreas in protein malnutrition (experimental kwashiorkor) and after cortisone administration. Amer. J. Path. **37**, 121–135 (1960)

Weisblum, B., Herman, L., Fitzgerald, P. J.: Changes in pancreatic acinar cells during protein deprivation. J. Cell Biol. **12**, 313–327 (1962)

Zeman, F. J.: Effect on the young rat of maternal protein restriction. J. Nutr. **93**, 167–173 (1967)

Prof. Dr. Klaus F. Wellmann
Isaac Albert Research Institute of the
Kingsbrook Jewish Medical Center
Brooklyn, New York 11203, U.S.A.