ULTRASTRUCTURAL BASIS OF
BIOCHEMICAL EFFECTS IN A SERIES OF
LETHAL ALLELES IN THE MOUSE

Neonatal and Developmental Studies

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ABSTRACT

The fine structure of newborn and fetal mouse liver and of newborn kidney cells homozygous for any of three albino alleles known to have multiple biochemical effects was investigated. Electron microscope studies of mutant cells revealed dilation and vesiculation of the rough endoplasmic reticulum in parenchymal liver cells, as well as dilation and other anomalies of the Golgi apparatus. These abnormalities were observed in all newborn mutants but never in littermate controls. Although they were most pronounced in liver parenchymal cells, they were found also to a lesser degree in kidney cells, but they were absent altogether in other cell types of the mutant newborn. Homozygous fetuses showed similar anomalies in the liver at 19 days of gestational age. In one of the alleles studied, mutant liver parenchymal cells were found to be abnormal as early as the 18th day of gestation. There appears to be a striking parallelism between the biochemical defects and those of the cellular membranes in homozygous mutant newborn and fetuses. Although the specific nature of the mutational effect on membrane structure remains unknown, the results are compatible with the assumption that a mutationally caused defect in a membrane component interferes with a mechanism vital in the integration of morphological and biochemical differentiation.

INTRODUCTION

In eukaryote systems cellular morphogenesis and biochemical differentiation are closely integrated, and both aspects of development are under genetic control. Therefore, not only structure of enzymes and proteins, but also their subcellular compartmentalization must be susceptible to mutational effects. An experimental system for the identification of normal mechanisms, operating in the integration of morphological and biochemical differentiation in higher organisms, may be provided by gene mutations causing simultaneously biochemical deficiencies and ultrastructural cellular abnormalities.

A series of radiation-induced lethal alleles at the albino locus in the mouse has been reported to cause multiple enzyme abnormalities. Homozygotes for any of four alleles studied die soon after birth and are deficient in glucose-6-phosphatase (Erickson et al., 1968). This enzyme begins to develop in normal mice during the last few days before birth. In lethal albino homozygous fetuses, lower than normal levels of glucose-6-phosphatase activity have been reported. Furthermore, recent additional studies of three of the homozygous mutants revealed deficiency of tyrosine aminotransferase in the newborn (Thorndike et al., 1973).

Since the mutational changes could not be ascribed to alterations in the structural genes for the respective enzymes, it became necessary to search for other causes.

The cellular and subcellular localization of the enzymes affected is known from cytochemical and biochemical studies. Glucose-6-phosphatase is normally found in the hepatic parenchymal cells, the renal proximal convoluted tubules, the epithelium of the intestinal mucosa, and the β-cells of the pancreatic islets of Langerhans. In all of these cells, the enzyme is located on the membranes of the endoplasmic reticulum (Leskes et al., 1971). Tyrosine aminotransferase is a soluble liver enzyme.

The multiplicity of morphological and biochemical defects caused by the mutant alleles and the microsomal localization of one of the affected enzymes raised the suspicion of possible ultrastructural membrane abnormalities in the mutant cells (Glueksohn-Waelsch and Cori, 1970). Consequently, electron microscope examinations of a variety of organs of newborn lethal homozygotes (c^{14CoS}, c^{112K}, and c^{3H}) and a developmental study of the fine structure of fetal mutant liver cells were undertaken.

MATERIALS AND METHODS

Mutant newborn mice were obtained from appropriate matings of the respective genotypes. The following genotypes were examined: (a) seven c^{3H}/c^{3H} and seven control littermates (four c^{ch}/c^{3H} and three c^{ch}/c^{ch}), (b) seven c^{112K}/c^{112K} and four control littermates, (c) eight c^{14CoS}/c^{14CoS} and four control littermates. Most homozygous lethal albinos die within a few hours of birth, and the majority of animals were estimated to be less than 6 h old at the time of fixation. But some c^{14CoS} homozygotes survive slightly longer, and on two occasions such newborn mice used in these studies were 10 and 12 h old. Where the exact age of animals is known, mention is made in legends to figures. All newborn mice

were killed by crushing the skull. The liver was exposed and small slices were removed, finely chopped, and fixed for 2 h in 2.5% glutaraldehyde—0.1 M cacodylate, pH 7.4, containing 6% sucrose. Samples were washed in 0.1 M cacodylate buffer with 12% sucrose for up to 1 h and postfixed in Millonig's osmium tetroxide. They were dehydrated through graded alcohols to propylene oxide and embedded in Epon 812. Sections were cut on a Reichert Om U2 ultramicrotome (C. Reichert, sold by American Optical Corp., Buffalo, N. Y.). Thick sections (1.0–2.0 μm) were stained in alkaline toluidine blue and examined by light microscopy. Thin sections were stained in alcoholic uranyl acetate followed by lead citrate (Reynolds, 1963) and examined in a Siemens Elmiskop 1A.

For the developmental studies, mutant and littermate control fetuses were used at 17, 18, and 19 days of gestation. The same genotypes served here as in the newborn studies. 7 albinos and 5 control littermates were examined at 17 days of gestation, 8 albinos and 9 controls at 18 days, and 10 albinos and 7 controls at 19 days. Matings were timed by the vaginal plug method. Pregnant females were killed by cervical dislocation and the uterus was quickly transferred to saline, where the fetuses remained alive for an extensive period. Individual fetuses were removed for fixation at approximately 10-min intervals. Liver samples were obtained and prepared in the same way as described above for the newborn.

For fixation of the kidney the thorax was opened and a 5 min ventricular perfusion with 2.0% glutaraldehyde was carried out through the heart. This kept open the lumina of the kidney tubules during fixation (Maunsbach, 1966). After a successful perfusion the kidneys were pale yellow in color and had a firm consistency. Poorly perfused kidneys had a reddish brown color, remained soft, and were not removed for study. Fixation by dripping glutaraldehyde or osmium tetroxide on to the exposed surface of the kidney was found to be ineffectual in mutant material, probably because these newborn became moribund extremely rapidly after surgical trauma. Perfusion also gives an effective preliminary fixation to many of the other internal organs. All organs

FIGURE 1   Control newborn liver parenchymal cell. Cisternae of rough endoplasmic reticulum RER, are arranged in regular parallel arrays. Golgi apparatus, G, consists of electron-opaque lamellae, small vesicles, and coated vesicles, CV. N, nucleus; M, mitochondrion. × 44,500.

FIGURE 2   Control newborn liver parenchymal cell. Endoplasmic reticulum cut to one side of cisternal lumina. Arrows indicate bound polysomes. × 44,500.

FIGURE 3 c^{112K}/c^{112K} albino newborn liver parenchymal cell. Endoplasmic reticulum. Note apparent disaggregation of bound polysomes, and loss of ribosomes on cisternal membrane (arrows). Traces of flocculent material are apparent within the cisternal lumen. My, myelin figure; ERL, endoplasmic reticulum lumen; I, inclusion. × 44,500.
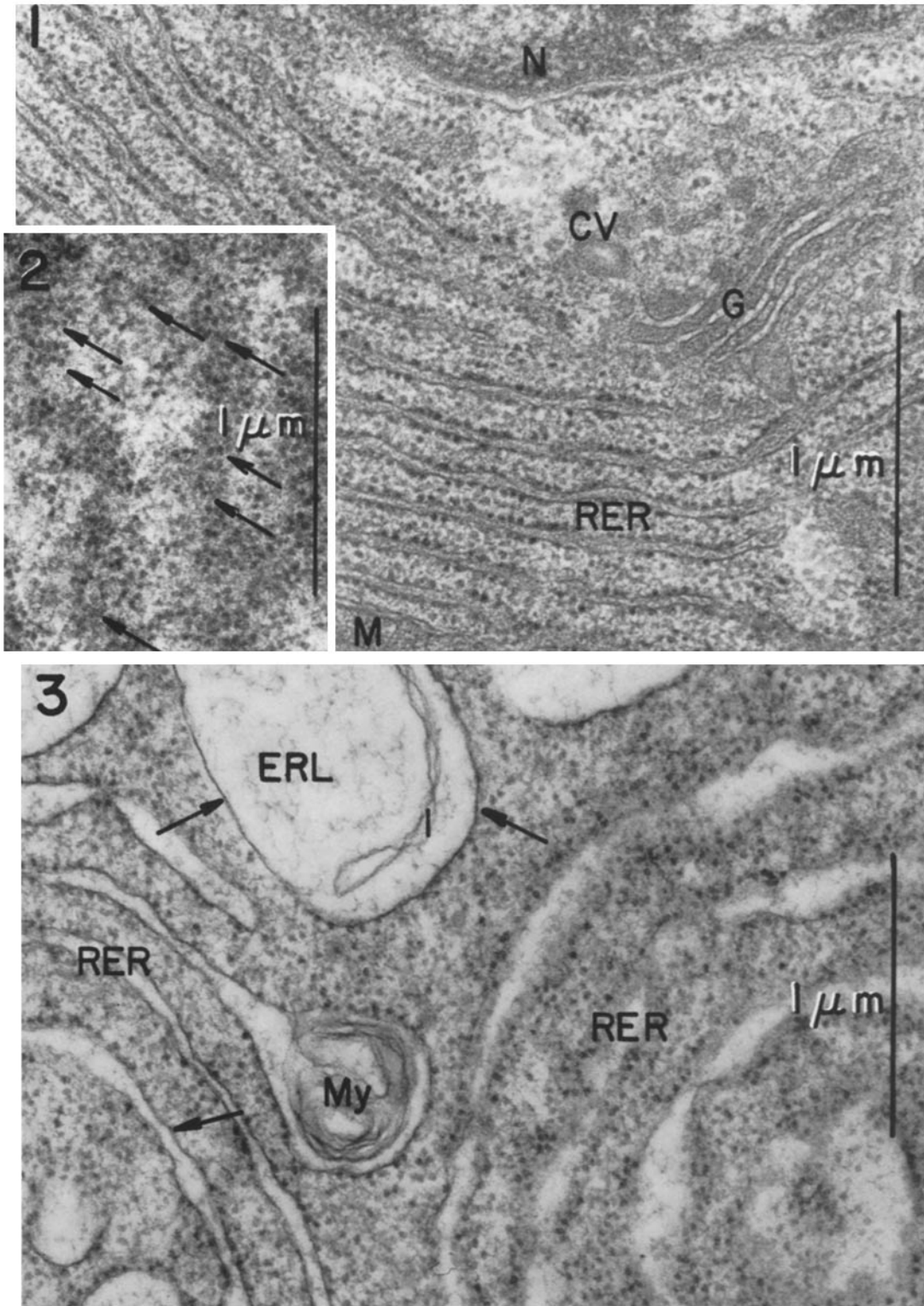
removed after perfusion were finely chopped and fixed for another 2 h in 2.5% glutaraldehyde— 0.1 M cacodylate, pH 7.4, containing 6% sucrose. Samples of kidney, small intestine, pancreas, adrenal gland, and thymus were obtained in this way. The procedure after glutaraldehyde fixation was the same as for livers.

RESULTS

Newborn Liver

Samples of normal liver from colored littermates of all three strains revealed no strain differences. In the c^{3H} strain the background pigmentation genes (a/a; b/b) allow heterozygous c^{ch}/c^{3H} animals to be distinguished from c^{ch}/c^{ch} homozygotes by eye color during late fetal and early neonatal life. Liver cells from these two genotypes showed no fine structural differences.

The absence of smooth endoplasmic reticulum and the presence of extensive glycogen deposits were the main features distinguishing parenchymal cells of the normal newborn from those of the adult. The fine structure of normal newborn liver has been described by Jezequel et al. (1965) and served as a basis of comparison here. Hemopoietic tissue, which constitutes a considerable proportion of the liver in newborn mice, showed no abnormalities in lethal albino homozygotes. Only the fine structure of parenchymal cells will therefore be reported here.

Rough Endoplasmic Reticulum

The regular arrangement of parallel cisternae characteristic of rough endoplasmic reticulum in parenchymal cells of the normal newborn (Fig. 1) was largely disrupted in mutant cells. In such
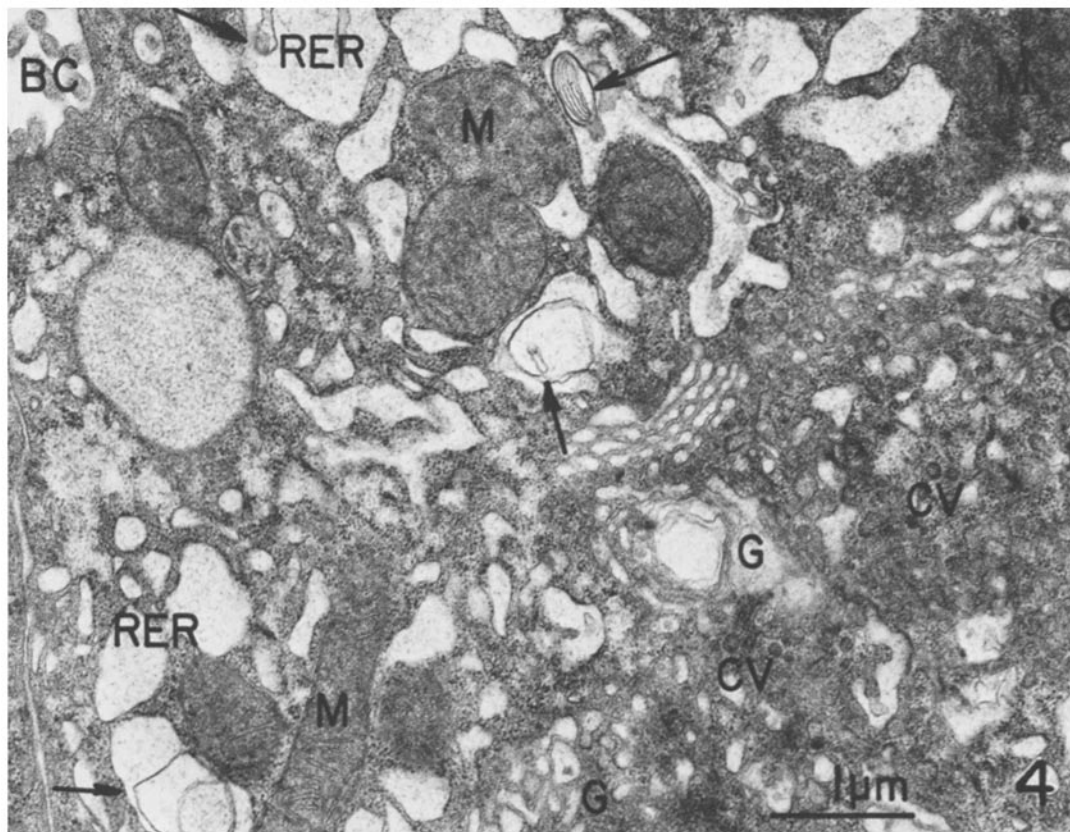

FIGURE 4 c^{14CoS}/c^{14CoS} albino liver parenchymal cell. Age 1 h after birth, showing mild to moderate abnormalities. Endoplasmic reticulum is apparent as dilated ribosome-studded vesicles and swollen cisternae, RER. Extensive electron-lucent Golgi lamellae showing slight dilation are present, G. Arrows indicate inclusions in swollen elements. Mitochondria in "orthodox" form, M. BC, bile canaliculus; CV, coated vesicle. × 13,300.

lethal albino parenchymal cells, the endoplasmic reticulum was consistently dilated or broken up into large vesicles (Figs. 4–5).

The loss of membrane-bound ribosomes was widespread where dilation was extreme, but focal loss of ribosomes and the disaggregation of polysomes were evident throughout the mutant endoplasmic reticulum (Fig. 3), while in normal parenchymal cells polysomal aggregates were easily visualized (Fig. 2). In the ground substance of the cytoplasm between the cisternae, free ribosomes were common.

Meandering endoplasmic reticulum cisternae or concentric circular configurations (Fig. 3) have been observed in samples from all three mutant homozygotes taken directly after birth and, also, in c^{14CoS} homozygotes some hours older. Large ribosome-studded vesicles (Fig. 4) characterized the parenchymal cells of all three mutants, and in c^{3H} and c^{112K} homozygotes this form of endoplasmic reticulum was predominant. Considerable dilation

was also observed in the nuclear envelope, which commonly retained close proximity to the nucleus only about the nuclear pores (Fig. 10).

Flocculent or amorphous material was usually present throughout dilated elements of the mutant endoplasmic reticulum or in a thin layer closely apposed to the cisternal membrane. Within the more dilated parts single and double membrane-delimited inclusion bodies (Figs. 4 and 5) were observed together with small fragments of membrane, myelin figures of variable size, and, very rarely, small fat droplets. It should be noted that ribosomes have never been observed in the included material nor attached to the inner surface of the membranes surrounding the inclusions.

The inclusions appear indicative of membrane lability, but they are not necessarily a characteristic feature of mutant cells in vivo. The particular method of fixation used (glutaraldehyde) does not exclude the possibility of their secondary origin from fixation effects.
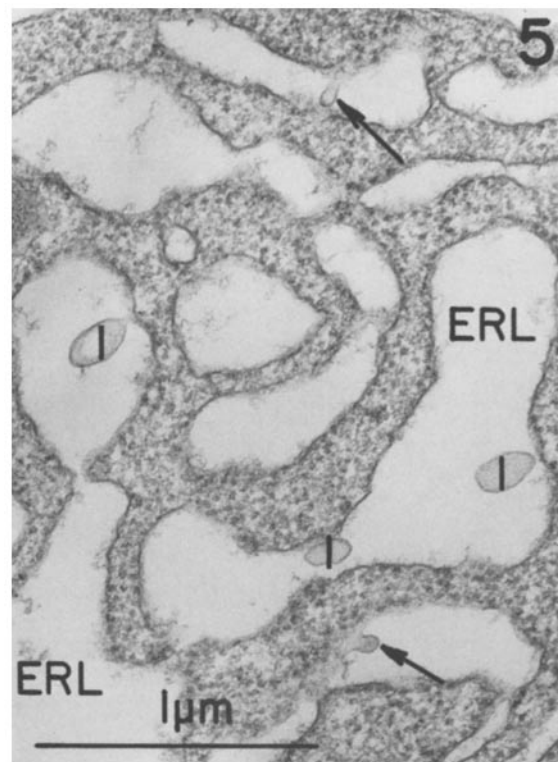

Figure 5 c^{14CoS}/c^{14CoS} albino newborn liver parenchymal cell. Inclusions I within elements of swollen endoplasmic reticulum. Arrows indicate formation of single membrane-delimited inclusions. Note distribution of flocculent material about the inner surface of the cisternal membrane and the predominantly electron-lucent nature of the vesicle lumina. ERL, endoplasmic reticulum lumen $\times$ 38,500.

Glycogen Deposits and Autophagic Vacuoles

Large amounts of stored glycogen were typical of normal newborn parenchymal cells (Fig. 6) while extensive depletion of glycogen deposits was evident in all mutant samples examined. In severely abnormal cells glycogen was absent altogether. In more mildly affected cells the segregation of glycogen masses into membrane-delimited compartments has been observed. This is probably preparatory to the hydrolysis of these masses by lysosomal enzymes. Autophagic vacuoles containing cytoplasm and free ribosomes or, rarely, glycogen were seen in some mutant cells in considerable numbers in contrast to controls where such activity was mild in comparison.

Golgi Apparatus

In mutant homozygotes the lamellae of the Golgi stack were swollen and lacked the electron-opaque granular content (Figs. 4 and 8), seen in the normal (Fig. 7). In rare cases cells had an abnormal endoplasmic reticulum and a normal Golgi apparatus, but the converse was never found. Dilation thus probably occurs first in the endoplasmic reticulum and then in the Golgi apparatus, but the two events follow very quickly upon one another.

In more severely affected cells extensive dilation was evident in all the Golgi lamellae (Fig. 9). The Golgi stack was represented by clusters of clear, irregularly shaped vacuoles, but the small vesicular elements of the normal Golgi apparatus could usually still be found. Lipoprotein granules, which form in small numbers from the control newborn Golgi apparatus (Fig. 7), were not found in mutant cells.

Small membrane-delimited inclusions, similar to those found in the endoplasmic reticulum, were occasionally observed in dilated Golgi elements. In addition, isolated bodies of cytoplasm were often found within dilated Golgi lamellae (Figs. 9, 10). These could occasionally be seen forming as indentations at the lamellar surface or intruding between swollen lamellae. The membranes of adjacent lamellae tended to retain a close proximity to one another even in instances of extreme dilation. The presence of dense cytoplasmic inclusions and the characteristic configurations of closely adjacent membranes allowed a tentative identification of many smooth membrane delimited vacuoles in the mutant cytoplasm as being of Golgi origin (Fig. 9).

Other Organelles

Mitochondria generally retained their normal morphology, but the condensed form was very common in mutant material (Fig. 10). In severely affected cells, only the condensed form was found and large multilobulate or oblong mitochondria could be observed. The number of peroxisomes appeared to decrease only in very abnormal cells. Such cells often lacked surface microvilli, which were sometimes seen internalized in autophagic vacuoles. Where the cytoplasm was extensively colonized by endoplasmic reticulum vesicles and other vacuoles, the nucleus was often deformed, indented, or elongated against the cell membrane. The nucleoplasm underwent no change except in the few necrotic cells observed, in which nuclear chromatin was clumped. Extensive areas of necrosis were never found in mutant livers, however, and individual instances of cell death were uncommon.

Extent of Abnormalities

Abnormalities were found in the entire parenchymal cell population in all mutant livers examined but never in those of normal littermates. There were, however, certain differences depending on the age and genotype of mutants (Figs. 4, 9, 10). In animals immediately after birth, abnormalities were least severe with less abnormal cells being scattered among those moderately and severely affected. At this stage c^{3H} homozygotes were the most abnormal and c^{112K} homozygotes somewhat less so. Within a few hours, however, mildly abnormal cells became uncommon and the two genotypes appeared to be affected with equal severity.

The least severe abnormalities were consistently observed in c^{14CoS} homozygotes. In these animals, which survive for a longer period after birth than homozygotes of the other two alleles, mildly affected cells were still found in samples 10 and 12 h old.

Normal Fetal Livers

Erythropoietic tissue constituted a large proportion of fetal livers, especially in younger samples. No abnormalities were observed in cells other than those of the liver parenchyma. Therefore, parenchymal cells only will be described here.

19-DAY OLD FETUSES: In 19-day old control fetuses, liver parenchymal cells showed large amounts of glycogen and the distribution of organelles was restricted to areas between glycogen
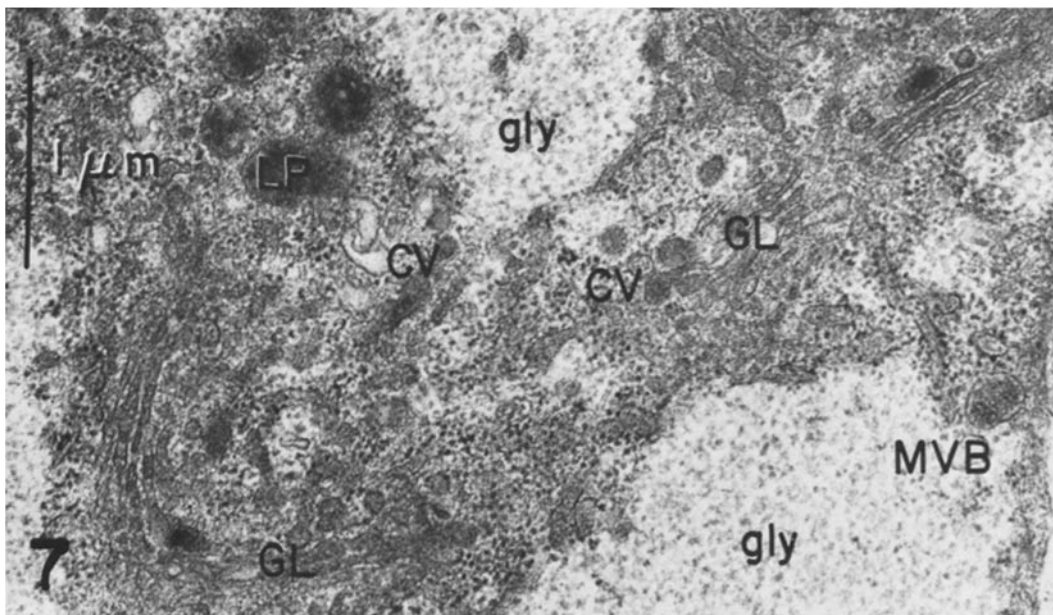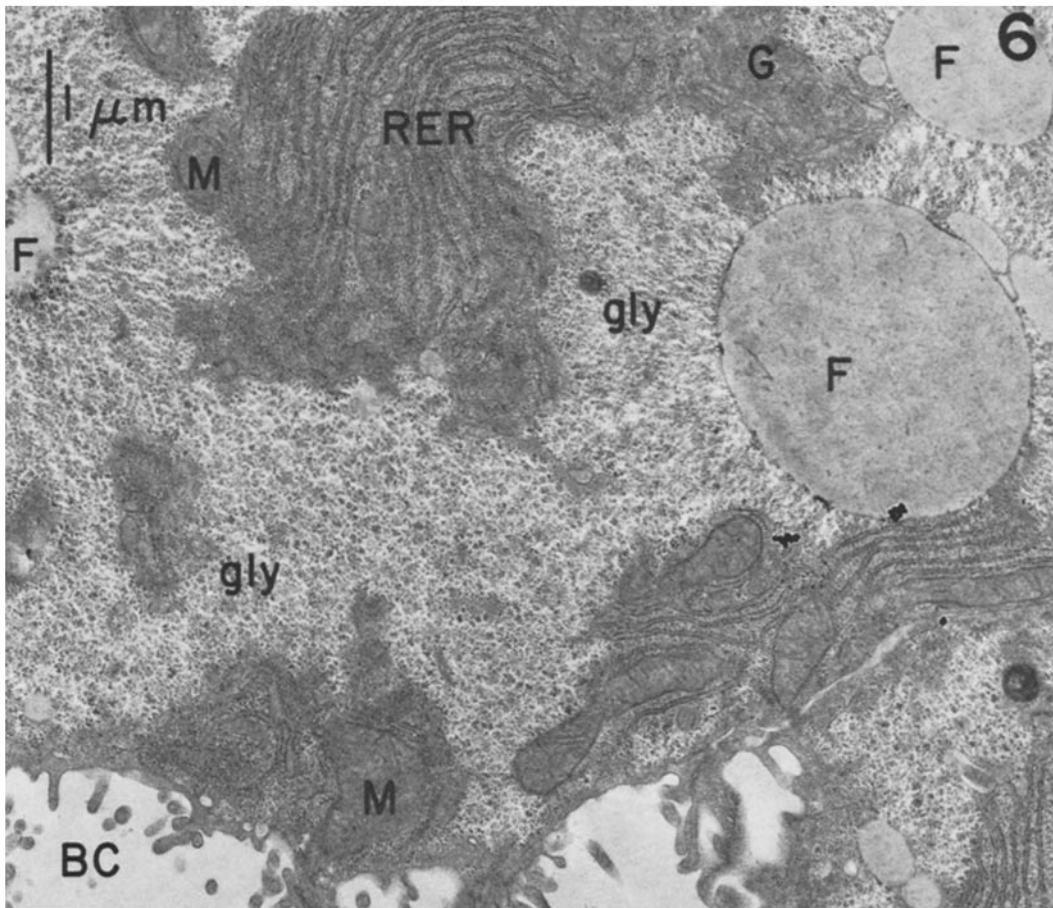
FIGURE 6   Control newborn liver parenchymal cell. Note extensive glycogen, *gly*, deposits. *F*, fat droplet; *M*, mitochondrion; *BC*, bile canaliculus; *G*, Golgi apparatus. × 14,000.

FIGURE 7   Control liver parenchymal cell. Normal Golgi apparatus. Note regular orientation of flattened Golgi lamellae, *GL; LP*, lipoprotein granules; *MVB*, multivesicular body; *CV*, coated vesicle; *gly*, glycogen. × 28,000.
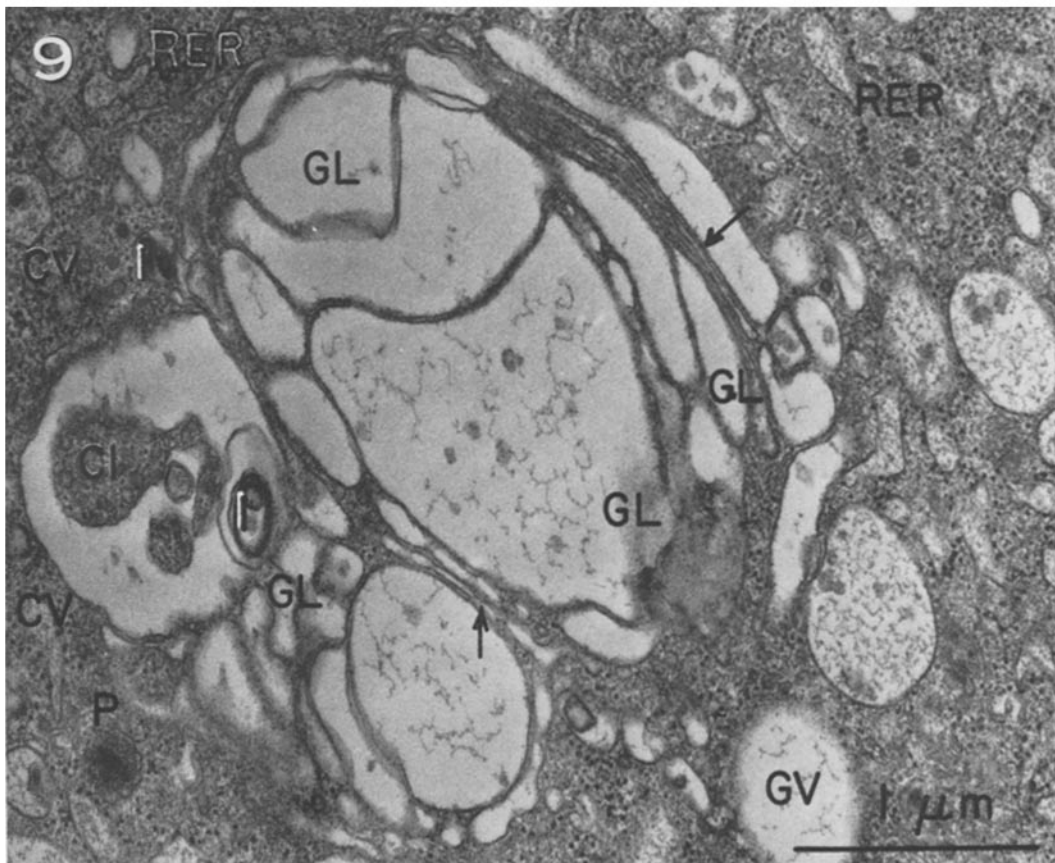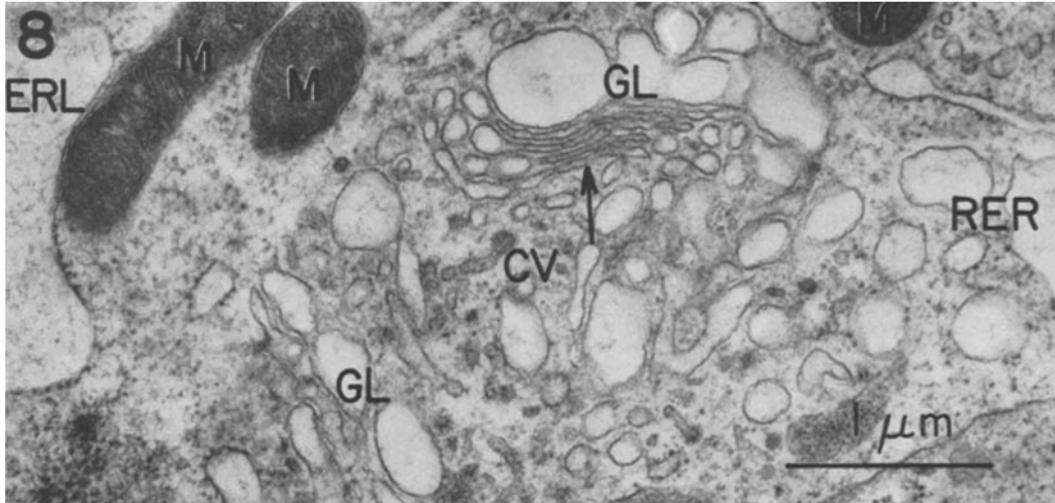
FIGURE 8 c^{14CoS}/c^{14CoS} albino liver parenchymal cell. Age 1 h after birth. Mildly dilated Golgi lamellae, *GL*. Arrow indicates more normal part of Golgi stack. *ERL*, endoplasmic reticulum lumen; *CV*, coated vesicle. Note electron-lucent content of all elements. $\times$ 28,000.

FIGURE 9 c^{112K}/c^{112K} albino newborn liver parenchymal cell. Golgi apparatus: there are few regularly oriented lamellae. The majority are extremely swollen, *GL*. Note close apposition of adjacent lamellar membranes (arrows). *CI*, cytoplasmic inclusion; *GV*, Golgi-derived vesicle; *P*, peroxisome; *CV*, coated vesicle; *I*, inclusion. $\times$ 28,000.

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deposits. The rough endoplasmic reticulum was, for the most part, arranged in orderly parallel arrays, while smooth endoplasmic reticulum was absent. The Golgi apparatus, situated in the perinuclear region, consisted of flattened lamellae, filled with electron-opaque material and many small vesicles (Fig. 11).
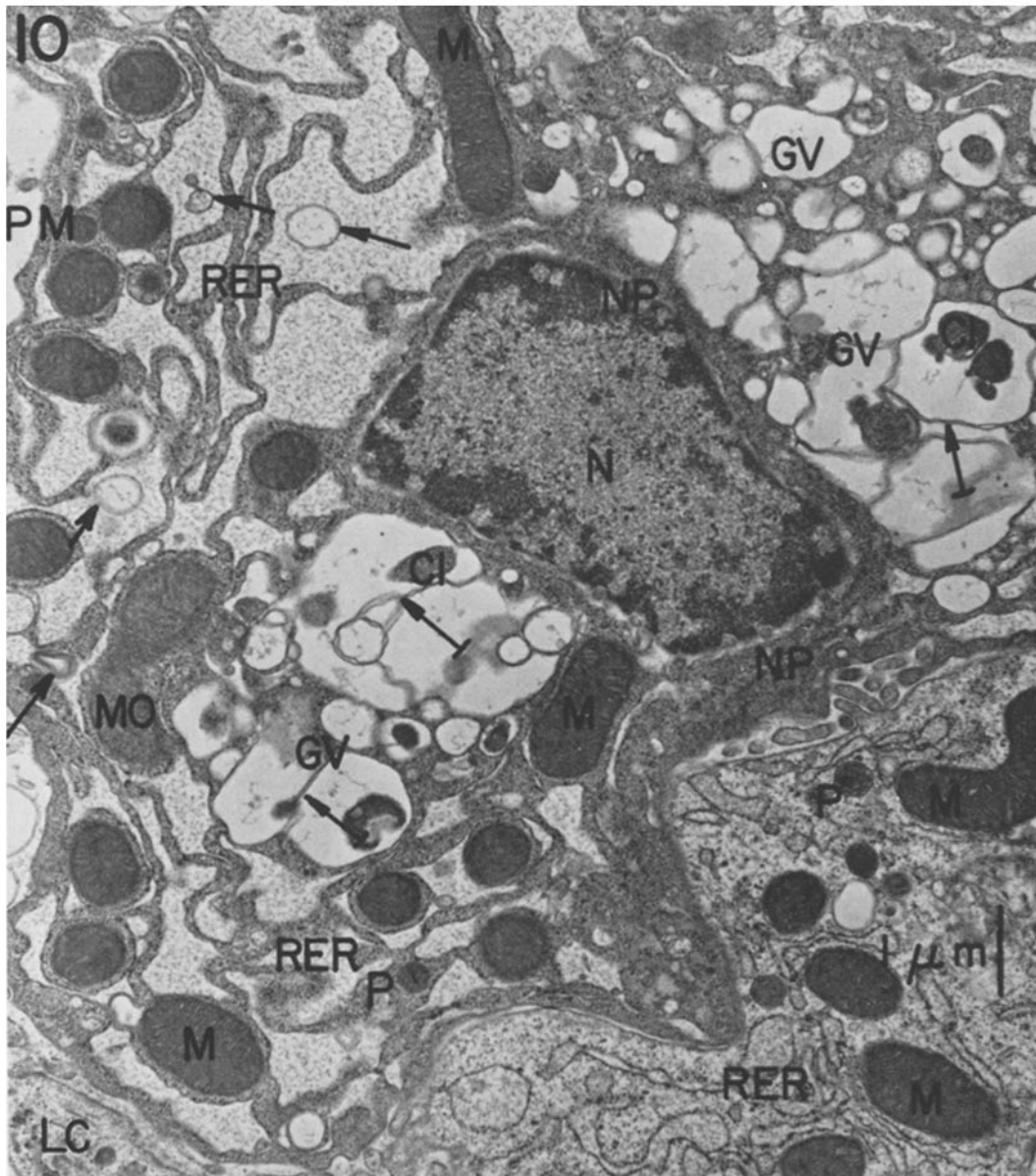
17 AND 18 DAYS OF GESTATION: Rapid

FIGURE 10 c^{3H}/c^{3H} albino newborn liver parenchymal cell. Dilation of endoplasmic reticulum is more pronounced than in Fig. 4. The numerous large vacuoles, GV, are probably of Golgi origin. Arrows indicate cytoplasmic inclusions in endoplasmic reticulum elements. Barred arrows indicate closely apposed membranes of adjacent Golgi vacuoles. A fine flocculent material is present within the dilated endoplasmic reticulum while Golgi vacuoles, GV, have a clearer content. Dilation is evident in the nuclear envelope, N, nucleus, except about the nuclear pores, NP. Note "condensed" mitochondria, $M;$ mitochondrion MO is in the "orthodox" form. Complete absence of glycogen. LC, sinusoid lining cell; CI, cytoplasmic inclusions; PM, plasma membrane; P, peroxisome. X 10,300.
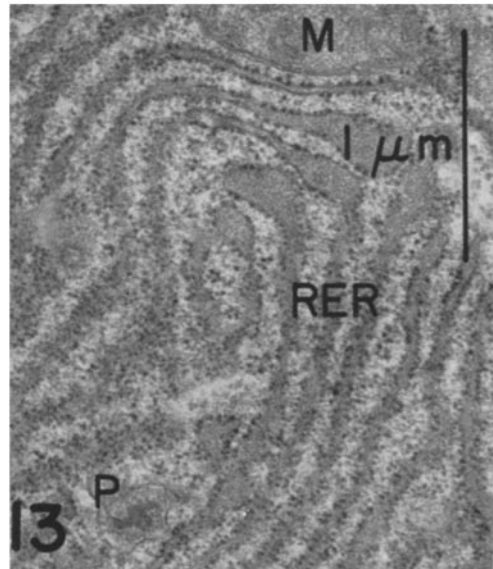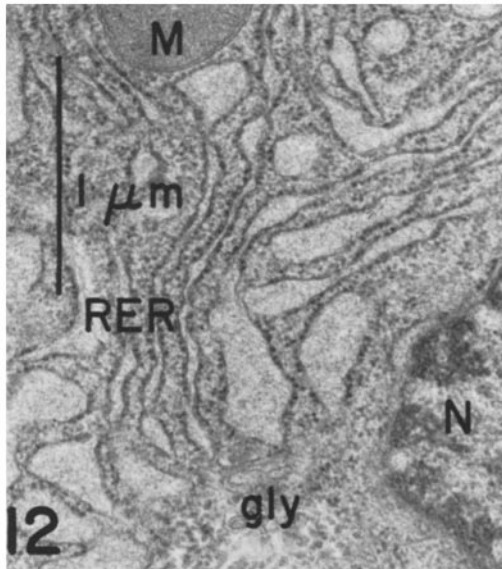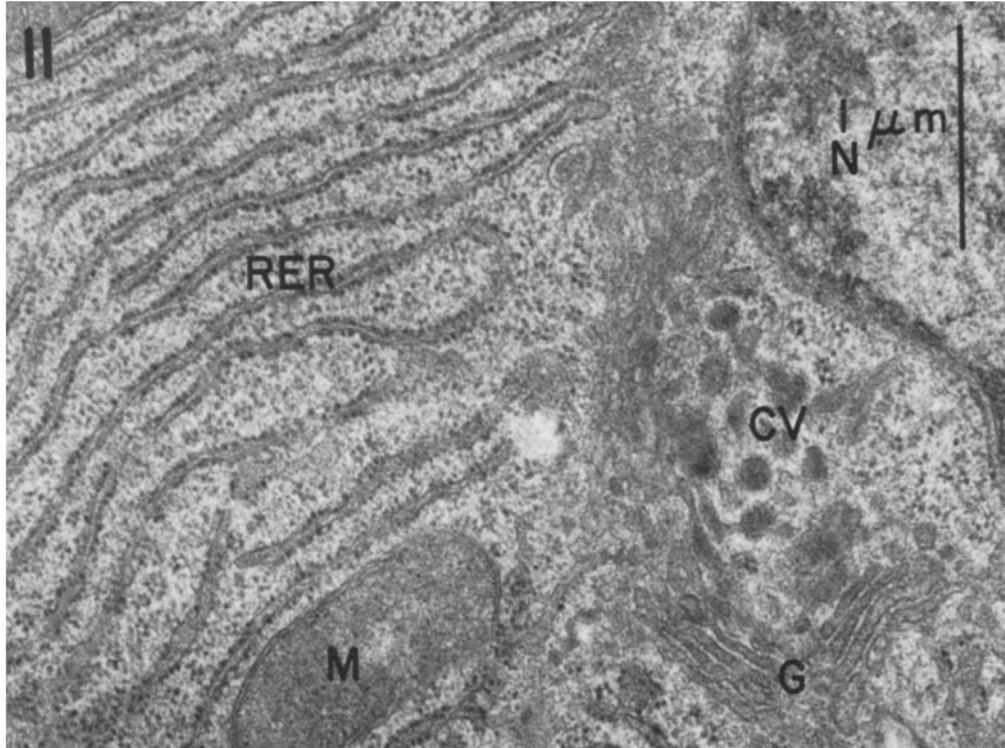
FIGURE 11   Parenchymal cell from 19-day old control fetus. *RER*, rough endoplasmic reticulum; *M*, mitochondrion; *G*, Golgi apparatus; *N*, nucleus; *CV*, coated vesicle. Note parallel arrangement of ER cisternae and electron-opaque content of Golgi lamellae. × 30,000.

FIGURE 12   Parenchymal cell from 17-day old control fetus. Note dilation of endoplasmic reticulum elements, *RER*, and irregularity of attachment of ribosomes to ER membranes. *gly*, glycogen; *N*, nucleus; *M*, mitochondrion. × 30,000.

FIGURE 13   Parenchymal cell from 18-day old control fetus. Note dilation of terminal parts of endoplasmic reticulum cisternae, *RER*. *M*, mitochondrion. *P*, peroxisome. × 30,000.

accumulation of glycogen was evident between the 17th and 18th day of gestation. The most striking differences between parenchymal cells from such fetuses and those of the 19 day old consisted in the state of relative disorganization of the rough endoplasmic reticulum in the younger fetuses. In 17-day old fetal parenchymal cells where there was little endoplasmic reticulum, the cisternae appeared widely dilated, and large ribosome-studded vesicles were commonly observed (Fig. 12). A more extensive endoplasmic reticulum was evident on the 18th day of gestation. Narrow cisternae were oriented in the more familiar parallel arrays, but they still terminated in large, saccate swellings, and large vesicles remained common (Essner, 1967)

(Fig. 13). Little change in the appearance of the Golgi apparatus was observed during this period.

Lethal Albino Fetal Livers

1 7 and 1 8 days of gestation : At 18 days of gestation, only c^{3H} homozygotes had a considerable number of abnormal cells. In these, dilation of endoplasmic reticulum and Golgi elements was found to be as severe as in many cells of older c^{3H} fetuses observed (Fig. 14). At the younger stage, such abnormal cells were observed scattered among other normal-looking cells.

In c^{112K} and c^{14CoS} homozygous fetuses of 18 days and in fetuses of all three genotypes at 17 days,
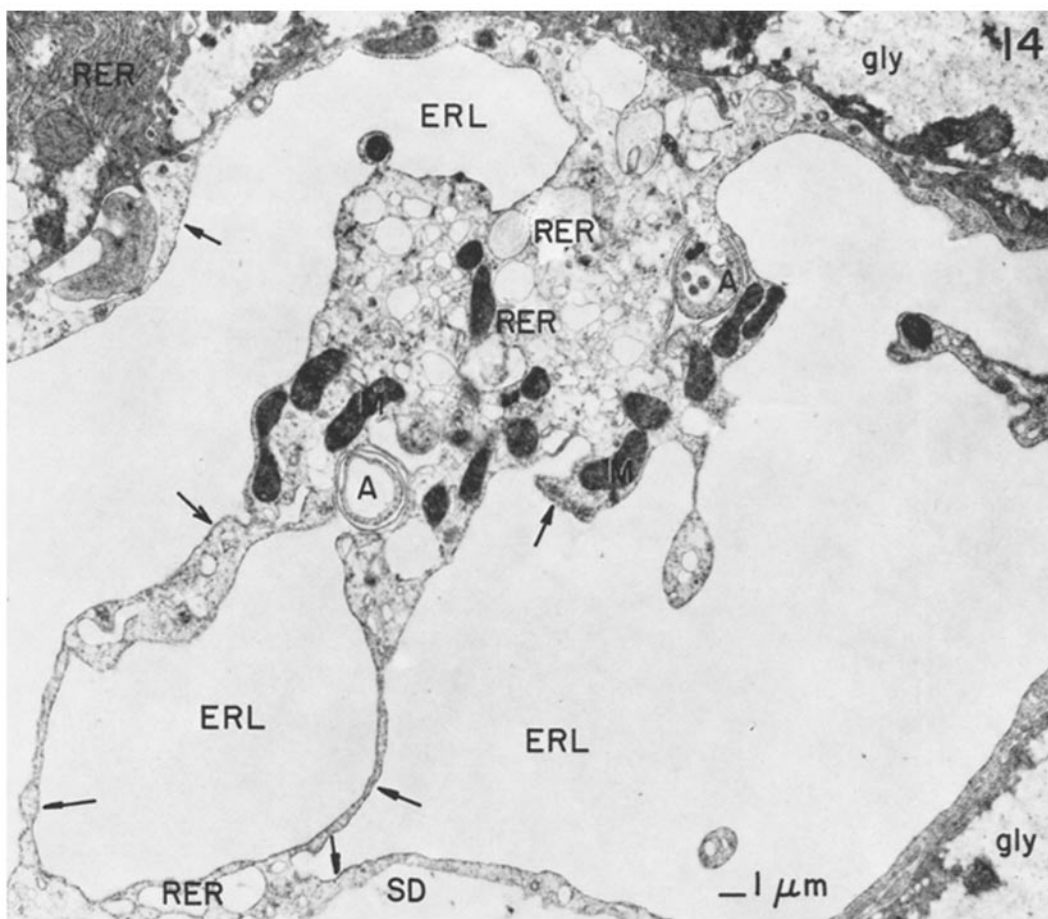

Figure 14 c^{3H}/c^{3H} albino parenchymal cell from 18-day old fetus. Note extreme dilation of endoplasmic reticulum. Arrows indicate bound ribosomes. Autophagic vacuoles, A, are forming from projections of the endoplasmic reticulum with ribosomes still present on the outer surface but missing from the inner surface. Mitochondria are in the condensed form. RER, rough endoplasmic reticulum; ERL, endoplasmic reticulum lumen; SD, space of Disse; gly, glycogen in adjacent normal-looking parenchymal cells. × 2,060.

abnormal cells were extremely rare. In the majority of mutant parenchymal cells the disoriented, vesiculated endoplasmic reticulum seen also in normal 17-day old fetuses was superseded at 18 days by the appearance of more regular, narrow parallel cisternae before the abnormalities characteristic of the lethal albino genotypes became apparent. Similarly, glycogen appeared to accumulate normally between the 17th and 18th day of gestation in the great majority of mutant parenchymal cells before depletion of deposits became evident.

19-DAY OLD FETUSES: Liver parenchymal cells from c^{14Cos} homozygotes showed considerable disorientation of the endoplasmic reticulum, with disaggregation of bound polysomes, and extensive but mild dilation. The lamellae of the Golgi apparatus were also commonly swollen. Small numbers of normal-looking cells were found in these samples. The majority of cells contained abundant glycogen deposits, in agreement with the biochemical data which showed normal glycogen values in fetuses (Erickson et al., 1968).

More severe abnormalities were always present in c^{112K} and c^{3H} homozygotes, in which normal-looking parenchymal cells were very rare at this age, in contrast to the mutant 18-day old fetuses. The endoplasmic reticulum was usually dilated particularly in c^{3H} homozygotes (Fig. 15). The extent of dilation in the endoplasmic reticulum exceeded that in normal 17-day fetuses and was often comparable with that found in newborn mutants. Swelling in elements of the Golgi apparatus was also evident, with loss of normal electron-opaque content, but the dilation was less extensive (Fig. 15) than in postnatal samples (Fig. 10). Inclusion bodies were occasionally seen in elements of both organelles. In the more abnormal cells glycogen deposits were somewhat depleted, but glycogen was very rarely totally absent. Depletion of glycogen in mutants around the time of birth is shown also by biochemical data (Erickson et al., 1968). Severely abnormal cells were scattered among cells with moderate and mild degrees of abnormality.

Newborn Kidney

In the kidney, glucose-6-phosphatase activity has been localized in the endoplasmic reticulum of proximal convoluted tubule cells (Kanamura,
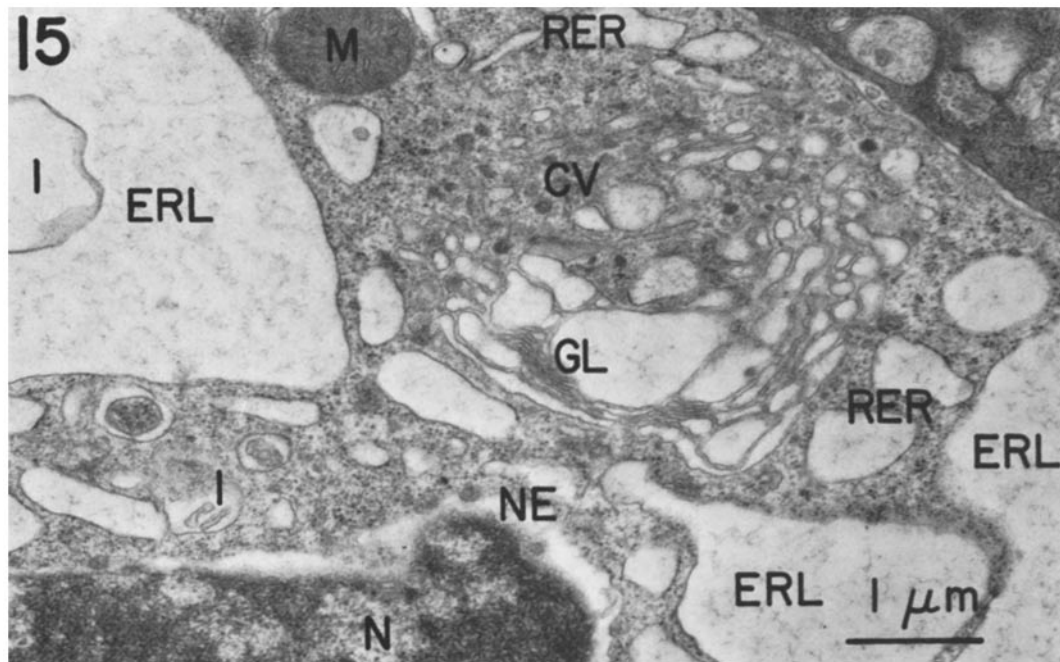

FIGURE 15 c^{3H}/c^{3H} albino parenchymal cell from 19-day old fetus. Note swelling in Golgi lamellae and loss of electron-opaque content, GL. Inclusions, I, are present in both endoplasmic reticulum and Golgi elements. RER, rough endoplasmic reticulum; ERL, endoplasmic reticulum lumen; N, nucleus; M, mitochondrion; CV, coated vesicle. Nuclear envelope, NE, also shows swelling. × 17,400.

1971). In mutant kidneys the activity of this enzyme has been shown to be diminished (Erickson et al., 1968).

In the normal control newborn the endoplasmic reticulum of the proximal convoluted tubules was composed of short tubular elements, mostly studded with ribosomes. Cisternae in oriented stacks were not found and the lumina of the endoplasmic reticulum profiles were commonly wider than those characteristic of liver parenchymal cells (Fig. 16).

In the proximal convoluted tubules of mutant kidneys no extensive abnormalities were found in the majority of cells. The organization of tubule cells and organelles other than the rough endoplasmic reticulum was apparently unaffected; in many cells the endoplasmic reticulum also looked normal. In some cells, however, especially in distal segments, large ribosome-studded endoplasmic reticulum vesicles were observed commonly basal to the nucleus, and were invariably filled with an electron-opaque granular material (Fig. 17). This abnormality of the endoplasmic reticulum was found in newborn kidneys from all three mutant homozygotes, but not in normals.

Intestine, Islets of Langerhans, Pancreas, Adrenal, Thymus

Other tissues in which glucose-6-phosphatase has been localized cytochemically are the intestinal mucosa (Hugon et al., 1971) and the β-cells of the islets of Langerhans (Lazarus and Barden, 1965). No abnormalities were observed in the endoplasmic reticulum, in any other organelles,

or in the general architecture of cells of duodenum, jejunum, and ileum from all three mutant homozygotes either directly after birth or after having been fed. Similarly in the islets of Langerhans, no evidence of abnormality was apparent.

The exocrine cells of the pancreas were examined as examples of cells with secretory function and an abundance of rough endoplasmic reticulum. The adrenal cortical cells were studied as cell types containing predominantly smooth endoplasmic reticulum. No abnormalities were detected in either.

In addition, the thymus was examined because of abnormalities of gross morphology reported in certain proportions of c^{3H} and c^{112K} homozygotes (Erickson et al., 1968); but here too no ultrastructural differences were observed.

DISCUSSION

The electron microscope studies of newborn mice homozygous for the lethal albino alleles c^{14CoS}, c^{112K}, and c^{3H} have identified extensive ultrastructural abnormalities of the endoplasmic reticulum and the Golgi apparatus in liver cells, and to a lesser extent in the kidney, whereas no such anomalies were observed in other cell types of mutants, or in any cells of normal littermate controls. The genetically caused defects of membrane structure may provide the common basis for the alterations of morphological and biochemical differentiation in these mutants.

Increased vesiculation and dilatation of the rough endoplasmic reticulum, as described here, have been reported as nonspecific responses to
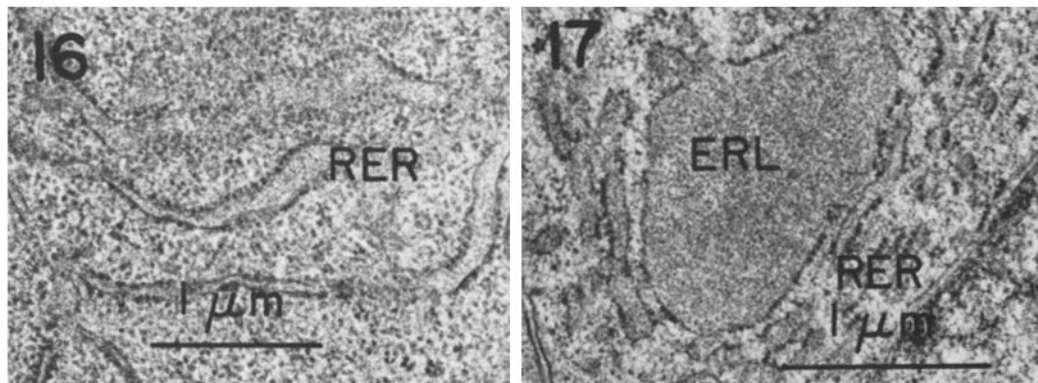

Figure 16 Control newborn kidney. Endoplasmic reticulum from proximal convoluted tubule cell. Note width and irregular orientation of endoplasmic reticulum elements, RER. × 28,000.

Figure 17 c^{3H}/c^{3H} albino newborn kidney. Endoplasmic reticulum from proximal convoluted tubule cell, RER. Note the large endoplasmic reticulum vesicle, ERL, with dense granular contents. × 28,000.

various types of cellular injury (Smuckler and Arcasoy, 1969). Nevertheless, the specificity of the ultrastructural defects in the mutants is suggested for several reasons. First of all, the degree of abnormalities and their presence in the newborn are unique. Secondly, the observation of similar ultrastructural abnormalities in mutant fetuses whose blood glucose levels had been shown to be normal (Glueksohn-Waelsch and Cori, 1970) excludes the possibility that the cellular anomalies in the newborn liver might represent secondary effects of hypoglycemia. Finally, the restriction of the defects to hepatocytes and kidney cells reflects the differentiation of specific properties of the membranes of these cell types as well as the specificity of the mutational effects.

The pattern of development of glucose-6-phosphatase, the enzyme characteristically deficient in these mutants, parallels certain aspects of the ultrastructural observations. In normal fetal rats, the enzyme develops in scattered cells of the parenchymal cell population a few days before birth, appearing simultaneously in all elements of the endoplasmic reticulum (Leskes et al., 1971); in older fetuses activity appears in more cells and in increasing amounts.

A scattering of affected cells also characterizes the pattern of ultrastructural abnormalities in c^{3H} homozygotes. There appears to be a parallel between the ultrastructural heterogeneity within the parenchymal cell population of 19-day old old mutant fetuses and the variations of cellular enzyme activity found on the day before birth by Leskes et al. (1971).

The different patterns of appearance of ultrastructural abnormalities in fetuses of the three mutant genotypes are reflected in similar variations of glucose-6-phosphatase activity (Thorndike et al., 1973). In both c^{14CoS} and c^{112K} homozygotes glucose-6-phosphatase fails to rise significantly after the 19th day of gestation. Thus, ultrastructurally abnormal cells make their appearance at the same time as levels of enzyme activity fail to rise. In c^{3H} homozygotes, abnormal cells become common on the 18th day, when the expected initial rise in enzyme activity fails to occur. Finally, the degree of abnormality on the cellular level is less extreme in c^{14CoS} homozygous fetuses, which have a significantly higher enzyme activity than the other two mutant types.

Although enzyme activity is abnormally low even during early fetal stages in c^{112K} and c^{14CoS} homozygotes, it appears likely that once dilation and the disaggregation of bound polysomes affect the endoplasmic reticulum a further rise in enzyme activity cannot occur either within the normal course of development or after the experimental administration of inducing agents (Thorndike et al., 1973).

There is parallelism also in the newborn between the genetically caused ultrastructural abnormalities and the biochemical effects of the lethal albino alleles reported earlier (Thorndike et al., 1973). Although qualitatively the defects of the rough endoplasmic reticulum and of the Golgi apparatus appeared identical in the three homozygous types (c^{14CoS}, c^{112K}, and c^{3H}), severity and extent of effects varied between genotypes. Whereas c^{14CoS} homozygotes showed the least abnormalities, those homozygous for c^{3H} were most severely affected. This is analogous to the biochemical deficiencies, particularly of glucose-6-phosphatase, which were most severe in c^{3H} and least so in c^{14CoS}. In spite of the qualitative similarity of their effects on biochemistry and on ultrastructure, the nonidentity of the alleles is indicated by their partial complementation with each other. Since they were radiation induced, they could be deletions of different sizes.

In an attempt to correlate the ultrastructural membrane defects with the biochemical deficiencies caused by these mutations, it appears relevant that glucose-6-phosphatase is bound tightly to the endoplasmic reticulum and is possibly even a membrane constituent. Membrane integrity might also be involved in the induction mechanism of tyrosine aminotransferase, an enzyme which is deficient and noninducible in these lethal homozygotes (Thorndike et al., 1973). This is indicated by the recent identification in newborn rats of a microsomal protein essential in the control of tyrosine aminotransferase induction at a translational level (Chuah et al., 1971).

Serum proteins, decreased in amount in lethal albino homozygotes, (Thorndike et al., 1973) are synthesized on the membrane-bound polysomes of the endoplasmic reticulum (Redman and Cherian, 1972) and normally leave the cell in membrane-bound Golgi vesicles. Decreased synthesis due to disaggregation of membrane-bound polysomes or decreased secretion by the abnormal Golgi lamellae might account for the serum protein deficiency in these mutants.

The observed depletion of glycogen deposits at a stage of mild membrane abnormalities is reflected in the biochemical data which in mutant homo-

zygotes show a decrease in liver glycogen at the time of birth (Erickson et al., 1968).

Although at present other possible interpretations cannot be excluded, the results reported here are compatible with the assumption that the lethal albino alleles affect a membrane constituent common to both the endoplasmic reticulum and the Golgi apparatus. Consequently, membrane integrity in homozygotes may be altered in such a way as to interfere with various vital membrane functions including the differentiation of certain membrane-bound enzymes.

The affected membrane component is likely to be a specific and essential part of liver microsomes, but of lesser importance in the kidney and not significant at all in membranes of other cell types which show normal fine structure in these mutants. The absence of a dosage effect in heterozygotes indicates that the action of one normal gene is sufficient to insure normal membrane development.

The existence of genetically caused ultrastructural defects in the endoplasmic reticulum and the Golgi apparatus during fetal development and in the newborn together with the multiple biochemical effects described earlier implicate these mutations in mechanisms integrating morphogenetic and biochemical differentiation. However, the precise nature of these mechanisms and of their constitutent parts remains to be determined.

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