Toxicity Testing of Fat Emulsions

II. Ultrastructural Changes in the Liver Following Administration of a New Intravenous Fat Emulsion (Intralipid)

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PIGMENT granules induced by intravenous fat emulsions have been studied by means of histochemistry[1-3] and electron microscopy[4] to elucidate their formation, nature and fate. Intralipid®, a new fat emulsion, has been said to cause less pigment deposition in experimental animals and considerably fewer reactions in man[5] than occurred with older preparations.[6] In the present study, livers of rats were examined via the electron microscope to ascertain pigment deposition and other fine structural changes of the hepatocytes occurring after the long-term administration of this emulsion.

PROCEDURE

The Intralipid used in the present study consisted of 1.2 per cent egg yolk phosphatide, 10 per cent soybean oil and 2.5 per cent glycerol.[5] Twenty-two rats were divided into three groups; each group was given fat emulsion or control substances intravenously via the tail vein for thirty successive days. The infusion of the rats in groups A and B was performed by Dr. H. C. Meng and Dr. T. Kuyama at the School of Medicine, Vanderbilt University, Nashville, Tennessee; and the infusion of the rats in group C was performed by Dr. S. W. Thompson and Dr. R. D. Hunt at the U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado. In group A, composed of eight rats, Intralipid was infused at a daily dose of 2 gm. per kg. body weight; two rats were killed on the day following the last dose, and three rats each were killed one and four and a half months after the last infusion. In group B, composed of eight rats, Intralipid was infused at a daily dose of 6 gm. per kg. body weight; two rats were killed one month following the last dose, and three rats each were killed one and a half to two months after the last infusion. The six rats in group C, which was to serve as the control were given infusions of 6 per cent dextran or physiologic saline solution at a daily dose of 10 ml. per kg. body weight. All rats in this group were killed on the day following the last infusion.

Liver tissue for electron microscopy was obtained by biopsy under light ether anesthesia. It was fixed in 1 per cent osmic acid, dehydrated in graded ethanol, embedded in Epon 812,[7] sectioned with a Porter-Blum microtome, stained with uranyl acetate[8] or lead citrate[9] and examined with a Philips EM-100 electron microscope.

At the time of necropsy, tissue specimens were collected from all major organs and tissues and were routinely fixed in 10 per cent neutral buffered formalin.[10] Specimens of liver tissue were also fixed in calcium formalin[10] for enzyme histochemistry.

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Paraffin embedded tissue sections were cut at 8 μ and stained with Harris' hematoxylin and eosin.[10] Serial paraffin embedded tissue sections of the liver and spleen, individually affixed to glass microscope slides, were subjected to those histochemical procedures which have been described as characterizing intravenous fat pigment.[1-4,10]

Frozen tissue sections, cut at 10μ in thickness, were prepared from the specimens of liver tissue fixed in calcium-formalin, and these were stained by Barka's naphthol AS-TR phosphate method for the demonstration of acid phosphatase activity.[10]

RESULTS

The results obtained in the study of conventional tissue sections, by means of bright field microscopy, have been described elsewhere.[11,12] Intravenous fat pigment deposits were observed in the reticuloendothelial cells of the spleens of all rats given Intralipid. However, only minute deposits of this pigment were observed in the livers of the same rats particularly in those given only 2 gm. of fat per kg. of body weight. All the histochemical procedures revealed changes in the pigment, both within the spleen and liver, to be similar to those previously described as being characteristic of intravenous fat pigment.[1-4,10] In addition, when observed within Kupffer cells, the pigment was associated with sites of acid phosphatase activity. Examination of the same tissues in rats given dextran or saline solution did not reveal any intravenous fat pigment nor any other significant lesion. The results described subsequently are related only to observations made by electron microscopy.

Group A. In the animals killed on the day following the last dose small pigment granules were found in the pericanalicular zone (Fig. 1). These granules measured 0.5 to 0.7 μ in diameter and possessed homogeneous or granular structures. Occasionally a single outer membrane surrounded them. However, distribution of pigment granules was different from cell to cell and some of the hepatocytes did not contain granules. The Golgi apparatus was prominent and was associated with dilated vacuoles containing moderately electron opaque material. Mitochondria and rough endoplasmic reticulum were usually normal, although some of the mitochondria were swollen and the rough endoplasmic reticulum displayed distorted parallel array. Microbodies were occasionally increased in number.

In the animals killed one month after completion of the infusions, pigment granules were larger, measuring 1.0 to 1.2 μ in diameter, and possessed a less electron opaque matrix with dense lobulated smaller globules (Fig. 2). Almost all the granules were surrounded by a single outer membrane. Slight dilatation of the rough endoplasmic reticulum was found. In those killed four and a half months after the completion of the infusions, the pigment granules were increased further in size, measuring up to 1.7 μ in diameter. The granules possessed variegated structures with various sizes of globules and electron lucent areas (Fig. 2, inset). The most remarkable change in this stage was dilatation of the rough endoplasmic reticulum

KEY TO ABBREVIATIONS IN FIGURES			
Bc:	bile canaliculus	P:	pigment granules
G:	Golgi apparatus	rER:	rough endoplasmic reticulum
M:	mitochondria		
N:	nucleus	Mb:	microbody

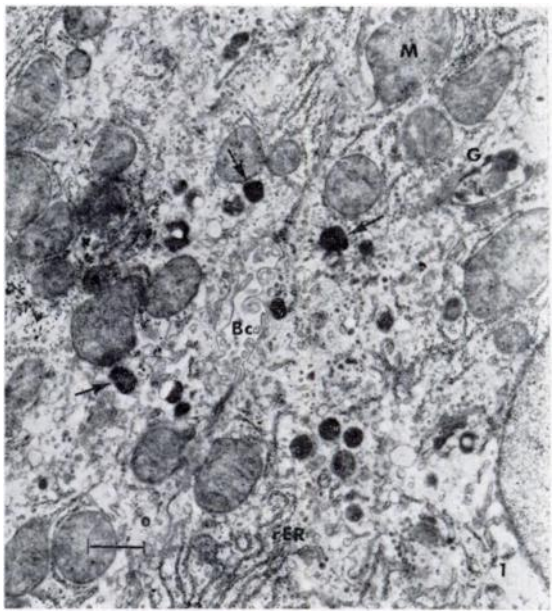

FIG. 1. Electron micrograph of liver of rat sacrificed on the day following the infusion (group A). Small pigment granules (arrows) are arranged peribiliarly and show moderate electron opaque matrix with smaller globules. Some of them are surrounded by a single outer membrane. The Golgi apparatus is prominent. Electron opaque materials are visible in the vacuoles and lamellae. Swollen mitochondria are also noted. Original magnification × 15,400.

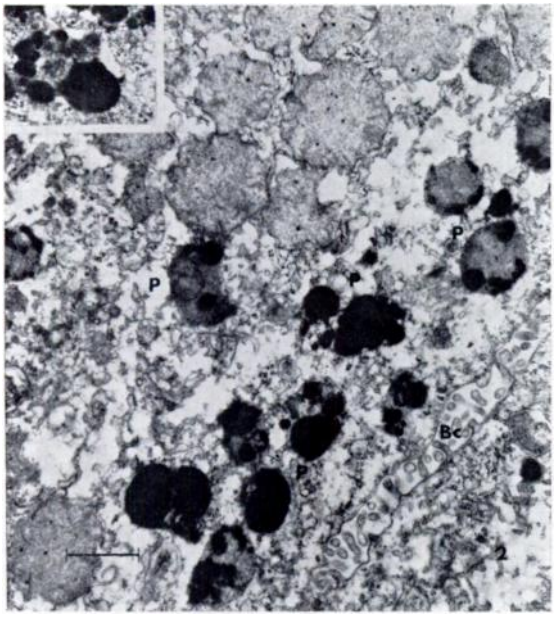

FIG. 2. The animal killed one month after the completion of infusions (group A). Pigment granules are increased in size. In the less opaque matrix dense globules with variegated shape and size are visible. Almost all of them are surrounded by a single outer membrane. Original magnification × 20,000. *Inset.* Rat killed four and a half months after the cessation of injections (group A). Pigment granules with aggregated matrix and globules. Electron lucent space is noticed in the periphery. Original magnification × 20,000.

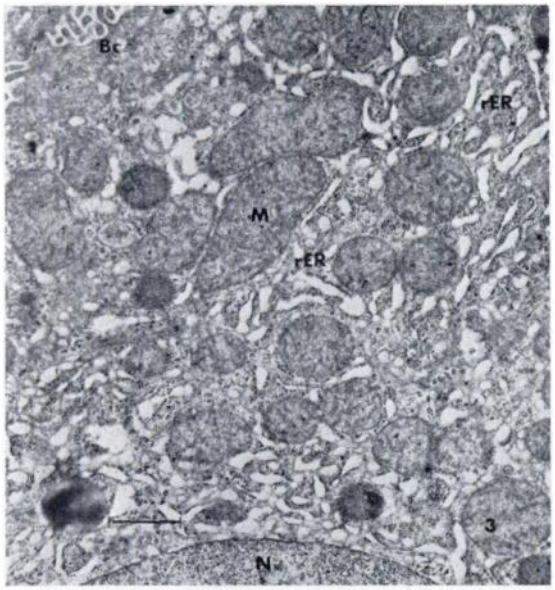

FIG. 3. Liver of rat sacrificed four and a half months after the last infusion (group A). Dilated rough endoplasmic reticulum with partial loss of ribosomes is scattered throughout the cytoplasm. Some of the mitochondria are swollen. The bile canaliculus is not surrounded by pigment. Original magnification × 20,000.

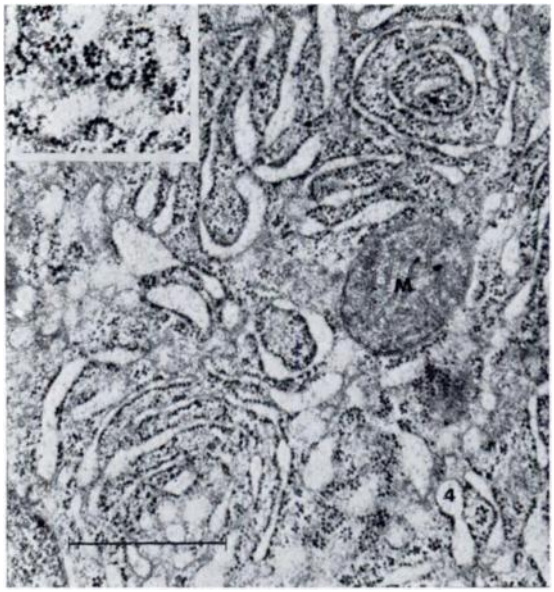

FIG. 4. Same animal as in Figure 3. Following the dilatation of rER, hyaloplasm forms thin rims, in which dislocated ribosomes are scattered. The concentric arrangement of the rER is visible. Original magnification × 46,000. *Inset.* Higher magnifications of ribosomes with a spiral arrangement and faint membrane attached to them. Original magnification × 74,600.

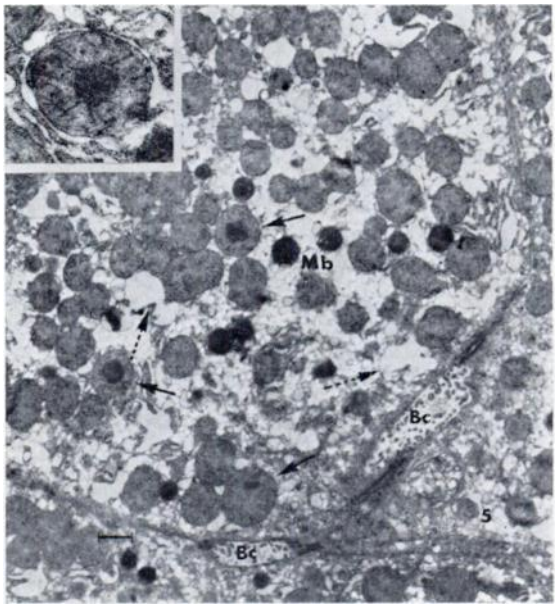

FIG. 5. Liver of rat killed one month after discontinuation of the injection (group B). Three mitochondria with dense central cores are visible (arrows). Microbodies are also increased in number. Cysternal dilatation of rER is detectable (broken arrows). Original magnification × 10,000. *Inset.* Higher magnification of a mitochondrion with core. No crystalline structures are visible. Mitochondrial cristae are directed toward the core. Original magnification × 20,000.

scattered throughout the cytoplasm (Fig. 3). A partial loss of ribosomes from the membranes of rough endoplasmic reticulum and a concentric arrangement of rough endoplasmic reticulum was also noted (Fig. 4). Free ribosomes were increased in number and occasionally had a helical or spiral arrangement, along which a faint membrane was visible (Fig. 4, inset). Microbodies were also increased in number.

Group B. The pericanalicular distribution, size and structures of the pigment granules in this group were essentially similar to those in group A. The rough endoplasmic reticulum was occasionally dilated, forming cysternal vacuoles with a partial loss of ribosomes as early as one month after the completion of the infusion (Fig. 5). A spiral arrangement of free ribosomes was also detectable in this stage. Microbodies were increased in number and a central core, measuring up to 0.6 μ in diameter, was found in some of the mitochondria (Fig. 5). In higher magnification, the central core possessed an aggregation of fine dense granules, but no crystalline structures (Fig. 5, inset). Mitochondrial cristae were directed toward the core. Two months after the completion of the infusions fat pigment became larger and dilatation of the rough endoplasmic reticulum was greater. No alterations of bile canaliculi were found in either group at any time.

Group C. A large number of dextran vacuoles 0.6 to 2.5 μ in diameter were found in the pericanalicular region. These vacuoles contained moderate opaque amorphous material or dense granules, resembling those described by de Man et al.[13] The rough endoplasmic reticulum as well as mitochondria were normal. No significant changes of organelles were observed in the animals given infusions of physiologic saline solution.

COMMENTS

The intravenous infusion of Intralipid produces three principle changes in hepatocytes, namely, pigment deposition, dilatation of endoplasmic reticulum and mitochondrial swelling. The pigment granules are around bile canaliculi and are small at first, measuring less than 1 μ in diameter and thus beyond the resolving power of the light microscope. The granules increase in size with age and may be twice as large months after the infusions have been discontinued than at the time the infusions were completed. In contrast, the large pigment granules induced by Lipomul® can be found after ten infusions and are detectable by conventional microscopy.[4]

Except for the differences in size, the fine structure of the pigment and the changes which occur with age are essentially the same with both emulsions. The chemical nature of the pigment has defied analysis to date, but its appearance and staining properties attest to its lipid character. A likely possibility is that it is a polymerized partially oxidized unsaturated fatty acid. These chemical alterations can and probably do occur in the emulsion itself, in the blood stream, in the liver or in the reticuloendothelial system.

A significant percentage of the intravenously infused lipid is taken up by the reticuloendothelial cells, the amount depending on the type of emulsion. Much of the pigment deposited or found in mesenchymal cells remains there but small amounts may be transported to the liver which is responsible for the growth of pigment granules after discontinuation of the infusions. In the hepatocyte, the infused lipid and any pigment already formed is taken up by pinocytosis.[14,15] The indigestible residue, such as pigment, is sequestered in lysosomes in the pericanalicular zone of the cell. Here with age it gradually loses its positivity to periodic acid-Schiff, and some of its acid phosphatase activity, suggesting some process-like increasing polymerization. It remains in the hepatocyte for the lifetime of the animal. Its appearance and staining qualities are those of lipofuscin,[16,17] or ceroid but not that of the granules in the Dubin-Johnson syndrome,[18,19] or hemosiderin.[16,20] The functional significance of the pigment granules remains to be established, although no evidence of long-term deleterious effects were noted in this study. The development of hepatocellular pigment *per se* should not be a contraindication to the use of an emulsion.

The changes which occurred in mitochondria seem to be dose related and, even when much larger doses of the lipid were given than recom-

mended clinically, were not striking. The mitochondrial core, resembling that seen in brown adipose tissue of hibernating animals,[21] may be chemically similar to the pigment. The relation of this to the dense granules normally found in mitochondria[22] is not known. Whether the increased number of microbodies reflects regeneration of mitochondria,[23] thereby indicating mitochondrial damage, requires further study. Mitochondrial degeneration is noted in experimental[24-26] as well as human[27] fatty livers in which altered mitochondrial function and impaired fat metabolism are related.

Dilatation of profiles of rough endoplasmic reticulum with dislocation of ribosomes from the membrane was present at the end of the infusion, increased somewhat in the subsequent month and persisted longer than four months. It was not the result of increased circulating fluid volume or of uptake of macromolecular suspension, since similar changes were not observed after infusions of saline solution or dextran. Dislocation of ribosomes in carbon tetrachloride intoxication is said to reflect impaired protein synthesis.[28] While similar changes of the endoplasmic reticulum can be produced by administration of phosphorus,[26] by viral infections,[29,30] and by experimental anoxia,[31] and are found in human fatty livers,[27] ribosomes usually also disappear concomitantly with hepatocellular degeneration.[24-26,29] Intravenous fat emulsions may be less effective anabolically than food, either because of the deficiency of protein or because metabolic pathways are altered by the excess of calories provided as fat. The latter in turn may use up enzymes or cofactors necessary in other metabolic processes. For instance a deficiency of choline may produce almost identical changes in the endoplasmic reticulum.[32] Increased numbers of spirally arranged ribosomes or polysomes have been presumed to be a sign of regeneration noted after administration of cysteine,[33] or alpha-naphthyl-isothiocyanate.[34] However, liver cell damage immediately following intravenous fat injections was not found in this or previous studies.[4] Therefore, the significance of the changes in endoplasmic reticulum cannot be evaluated, particularly since intravenous fat emulsions are to be used in depleted patients in whom maximal protein synthesis is needed.

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