ISOLATION AND CHARACTERIZATION OF LOW DENSITY STRUCTURES FROM OROTIC ACID-INDUCED FATTY LIVERS

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ABSTRACT

Centrifugation of a sucrose homogenate of the livers of female albino rats fed a 1.5% orotic acid diet for 3 wk yielded a pellicle containing low density structures. In morphology and biochemical properties these structures resembled those portions of endoplasmic reticulum which accumulated lipid. Electron microscopy indicated large droplets of lipid bounded by a membrane with attached ribosome-like particles. The presence of ribosomes in these structures was established by treatment with deoxycholate and centrifugation. The proportion of 18S and 29S RNA was the same as that found in the ribosomes from normal liver; however, the distribution of radioactivity between the 18S and the 29S RNA after injection of 8-^{14}C-adenine was distinctly different. The RNA isolated from these structures contained a higher guanylic acid to cytidylic acid ratio than that found in the microsomes of the normal liver. It is proposed that these low density structures may be those portions of the endoplasmic reticulum in which there exists a defect responsible for the block in the assembly or secretion of plasma lipoprotein.

INTRODUCTION

The occurrence of fatty livers in albino rats fed a purified diet supplemented with 1% orotic acid was observed as early as 1955 by Standerfer and Handler (1). The report of Handschumacher et al. (2) on the prevention of this fatty infiltration by adenine suggested that the accumulation of lipid was related to an imbalance in the purine and pyrimidine nucleotides. Subsequent studies indicated that most of the lipid was triglyceride and that the plasma lipids were reduced (3–6). The normal accumulation of plasma lipid after administration of Triton 100 was greatly diminished in animals fed the orotic acid diet (7). Associated with these changes was a decrease in the total amount of adenine nucleotides, an effect which could be prevented by the inclusion of adenine in the diet (8, 9). All of these effects are similar to those produced by the antimetabolite ethionine.

The deposition of triglycerides in the liver accompanied by a decrease in the concentration of plasma lipids is a common and characteristic feature of fatty livers induced by CCl_4 (10), puromycin (11), ethionine (12), azaserine (13),

4-amino-pyrazolopyrimidine (14, 15), orotic acid, and a diet deficient in choline. The basic mechanism underlying the pathogenesis of fatty livers induced by the compounds mentioned above seems to be a block in the release of triglycerides into the plasma. Since triglycerides are released from the liver into the plasma in the form of lipoproteins it appears that the primary defect in this group of fatty livers is either the synthesis or the secretion of the lipoproteins, or both. Recent studies by electron microscopy of the fatty livers caused by orotic acid, CCl_4, ethionine, and choline deficiency (16–21) have revealed the appearance of osmiophilic bodies within the endoplasmic reticulum. The osmiophilic bodies appearing in the livers of rats after the administration of a diet containing orotic acid were found to be lipid in nature and were associated with the endoplasmic reticulum (16, 17).

Since the appearance of lipid in the endoplasmic reticulum is considered to be the earliest morphological expression of a block in the release of lipoproteins from the liver (20), the isolation of the morphologically affected regions of the endoplasmic reticulum would provide a valuable tool for studying the mechanism of fatty liver production. The use of orotic acid for this purpose offers a distinct advantage since this pyrimidine precursor causes no inhibitory effect on general protein synthesis (22–24) and does not grossly alter the general architecture of the liver (16).

The observation by Rajalakshmi et al. (25) that a floating material which primarily contains lipid admixed with significant amounts of RNA and protein could be isolated by centrifugation of the homogenate from orotic acid–induced fatty livers served as the basis for the current work. The present report is concerned with the isolation and characterization of this material which in morphology and biochemical properties is very similar to the affected regions of the endoplasmic reticulum in the whole liver.

MATERIALS AND METHODS

For electron microscopy, all specimens were fixed for 8 to 24 hr in a buffered 2% solution of glutaraldehyde containing 3% glucose, 0.1 M sodium cacodylate, pH 7.4. They were treated for 1 hr in 1% OsO_4 in the same buffer and embedded in Epon (26). Thin sections were cut with a glass or diamond knife on a Sorvall model MT-2 ultramicrotome, stained with uranyl acetate–lead citrate (27), and examined in a Siemens Elmiskop I. Micrographs were taken at instrument magnifications of 8,000 to 40,000 and enlarged photographically to the values stated in the figure captions.

Female albino rats (50–60 g) of the Sprague-Dawley strain (Charles River Breeding Laboratories, Wilmington, Mass.) were housed in indiviudal cages and fed ad lib. The rats of the control group received the basal purified diet (2) supplemented with 1.5% orotic acid for a period of 3 to 4 wk. At the end of the experimental period, the rats were killed by decapitation and the livers were chilled immediately in medium A (0.25 M sucrose, 0.0033 M $CaCl_2$, 0.05 M tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.4). All operations were performed with solutions at 0°C. The livers were weighed quickly, minced with scissors, and homogenized in medium A (9 ml/g liver) with five strokes of a Teflon-glass Potter-Elvehjem homogenizer in an ice bath. This homogenate was used for the preparation of microsomes, ribosomes, and "low density structures."

The homogenate was centrifuged at 105,000 *g* in a Spinco model L with a No. 40 rotor for 1 hr, and the thick white fatty pellicle which floated on the top of the supernatant fraction was removed with a spatula. This material was carefully washed by redispersing in a beaker at 0°C with medium A to remove the supernatant fraction adhering to the material and was resuspended in medium A (20–25 ml, for the material recovered from one liver) by gentle homogenization. After centrifugation at 105,000 *g* for 30 min in a No 40 Spinco rotor, some particulate material sedimented to the bottom of the tube and a pellicle remained at the top of the sucrose solution. This floating material was used for the preparation of electron micrographs. Most of the biochemical work reported in this communication was done with this material. Centrifugation of this material after rehomogenization in medium A did not yield sedimentable material.

The postmitochondrial supernatant fraction was prepared by centrifuging the liver homogenate described above for 20 min at 10,000 *g*. The upper ⅔ of the supernatant fraction was carefully removed without disturbing the layer of lipid on the top or the sediment. The microsomes were sedimented from this solution by centrifugation at 105,000 *g* for 1 hr. Ribonucleoprotein particles for morphological study were prepared by the method described by Ernster et al. (28) except that 0.5% deoxycholate was used.

For the preparation of ribosomes, the postmitochondrial supernatant fraction was prepared in medium B (0.25 M sucrose, 0.025 M KCl, 0.005 M $MgCl_2$, 0.05 M Tris-HCl, pH 7.6) by the method described above. To 10 ml of this solution, 1.5 ml of a 10% deoxycholate solution was added with stirring and the mixture was centrifuged for 2 hr at 105,000 *g*. The ribosomes from the fatty material isolated from the orotic acid–induced fatty liver were prepared in a similar manner.

For the preparation of RNA, the ribosomes, microsomes, and the fatty material were homogenized at 4°C in 4 volumes of Tris-HCl buffer (0.1 M, pH 7.6) containing 1% sodium dodecyl sulfate. An equal volume of phenol (analytical grade, Fisher Scientific Co., Pittsburgh, Pa.) saturated with water was added, and the mixture was stirred with a magnetic stirrer for 45 to 60 min at 5°C. At the end of the extraction period, the solution was centrifuged at 10,000 *g* for 20 min. The aqueous layer was removed and adjusted to a concentration of 0.1 M NaCl and 0.01 M sodium acetate, pH 5.1. The RNA was precipitated by the addition of 2.5 volumes of absolute ethanol and was washed repeatedly with ethanol to remove phenol. The final precipitate of RNA was dissolved in 0.1 M NaCl containing 0.01 M sodium acetate, pH 5.1.

For the sucrose density gradient analysis, the RNA isolated from the ribosomes (6–8 $A_{260\ m\mu}$ units in a volume of 0.6 ml) was layered on a 50 ml linear sucrose gradient (5–20%) prepared in a solution of 0.1 M NaCl containing 0.01 M sodium acetate, pH 5.1, and centrifuged for 20 hr at 20,000 rpm in an SW 25.2 rotor in the Spinco model L2-65. After centrifugation, the sucrose solution was drawn from the bottom of the tube with a Buchler pump at a rate of 1.5 ml/min and passed through a flow cell (Beckman DB) and a recorder (Beckman) for measurement of absorbancy at 260 mμ. Fractions were collected at 1-min intervals, 50 μg of albumin were added, and the RNA was precipitated with 0.4 N perchloric acid at 4°C overnight. The precipitate was collected on Millipore filters, washed with 0.4 N perchloric acid, and the radioactivity on the membrane filters was determined in glass vials containing 10 ml of a toluene-ethanol scintillation fluid.

For the analysis of base composition, the RNA isolated from the low density structures was hydrolyzed with 0.3 N KOH at 37°C for 18 hr. The nucleotides were adsorbed at pH 8 on a 20 × 1 cm column of Dowex 1 × 8 (200–400 mesh, formate form). The nucleotides were separated by gradient elution with 500 ml of 4 N formic acid in the reservoir and 500 ml of water in the mixer. Fractions of 6 ml were collected and the absorbancy at 260 mμ and 280 mμ was determined. Each peak was pooled and the concentration of the nucleotides was calculated by using $E_{260\ m\mu}$ for adenylic acid of 14.2×10^3; for guanylic acid of 11.8×10^3; for uridylic acid of 9.9×10^3; and $E_{280\ m\mu}$ of 12.7×10^3 for cytidylic acid.

Since the morphology of the low density structures resembled that of the endoplasmic reticulum, the following experiment was performed to determine whether the ribosomes found in this material were merely entrained by the lipid layer. 8-^{14}C-adenine (10 μc) was injected intraperitoneally into a rat from the control group 24 hr prior to killing. 1 g of the liver from this animal was homogenized in medium A with an equal weight of liver from a rat fed an orotic acid diet for 3 wk. The pellicle of low density structures was isolated from this mixed homogenate by the method described earlier. The nucleoprotein was precipitated with 70% alcohol containing 10% trichloroacetic acid at 4°C. The precipitate was washed three times with 5% trichloroacetic acid to remove acid-soluble nucleotides, once with alcohol saturated with potassium acetate; four times with alcohol:ether (3:1), and finally with ether. The nucleoprotein was hydrolyzed with 2 ml of 0.3 N KOH at 37°C for 18 hr, the pH was adjusted to 2 with perchloric acid at 0°C, and the precipitate was removed by centrifugation. The absorbancy at 260 mμ and the radioactivity were determined, and the percentage entrainment was calculated from the specific radioactivity of the RNA in the low density structures compared to that of the normal microsomes. A similar experiment was performed with a portion of a radioactive liver from a rat fed the orotic acid diet and unlabeled liver from the rat in the control group. In addition, an experiment was performed to determine whether lipid alone can entrain particulate material. Lipid from orotic acid–induced fatty liver and the low density structures was isolated by the method described previously (2). 1 gram of radioactive liver from the rat belonging to the control group was mixed with 300 mg of lipid. A floating material was isolated by the same method employed for the isolation of the low density structures described earlier.

RESULTS

A comparison of the electron micrographs of sections from normal liver (Fig. 1) and livers from animals that had been fed the orotic acid diet for 3 wk (Fig. 2) reveals changes qualitatively similar to those reported after 10 days by Jatlow et al. (16). These changes include a general dilatation of the endoplasmic reticulum with some apparent depletion of attached ribosomes. These dilatations coalesce to form larger vacuoles. A similar dilatation of the reticulum and fusion of dilated segments has been observed in the livers of animals treated with ethionine (20).

After homogenization and separation of the cellular components by centrifugation, these differences in the animals fed the control diet and the orotic acid diet could be identified in the electron micrographs of microsome preparations (Figs. 3 and 4), particularly in the low density structures from rats fed orotic acid (Fig. 5) which form a pellicle during centrifugation. In the preparation of low density structures a large number of vesicles bounded by membranes can be seen together with minor contamination by mitochondria and other cellular

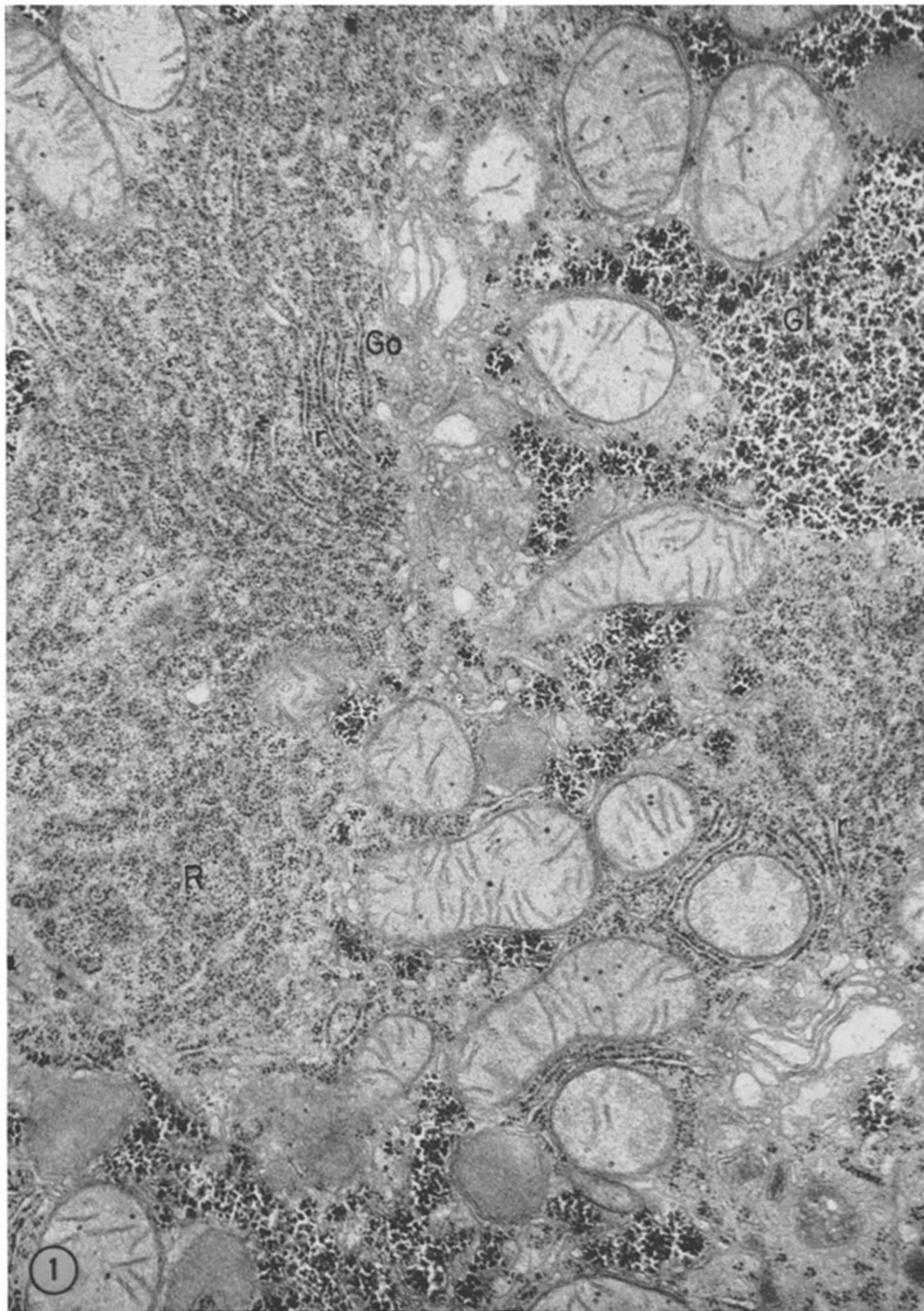


FIGURE 1 Rat liver (normal diet). Mitochondria are uniform. Golgi material (Go) is within normal limits. Rough endoplasmic reticulum (r) shows no tendency to dilatation and contains a uniform encrustation of ribosomal particles. Moderate quantities of glycogen (Gl) are present. Numerous free ribosomes are visible (R). No lipid vacuoles are seen. × 24,000.

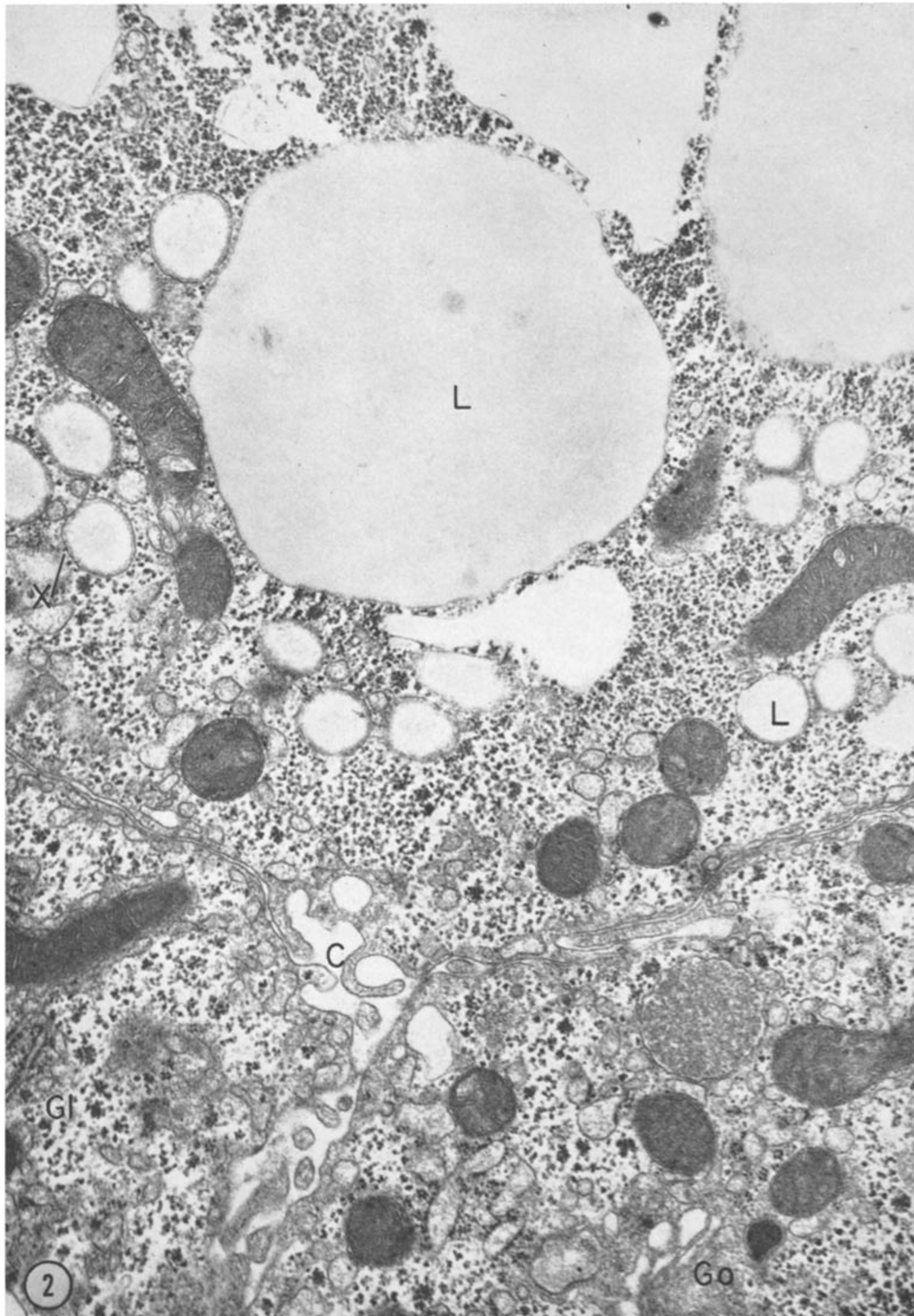


FIGURE 2 Rat liver (orotic acid diet). Mitochondria are uniform but slightly smaller and more dense than in the control. The significance of the latter observations is not apparent. A canaliculus (C) lies at the juncture of three cells. Golgi material (Go) is relatively unchanged. Dilated sacs (L) of rough endoplasmic reticulum, identified by occasional membrane bound ribosomes (as at X), contain faintly osmiophilic material interpreted as lipoprotein. Glutaraldehyde-osmium tetroxide fixation, poststained with uranyl acetate-lead citrate. × 24,000.

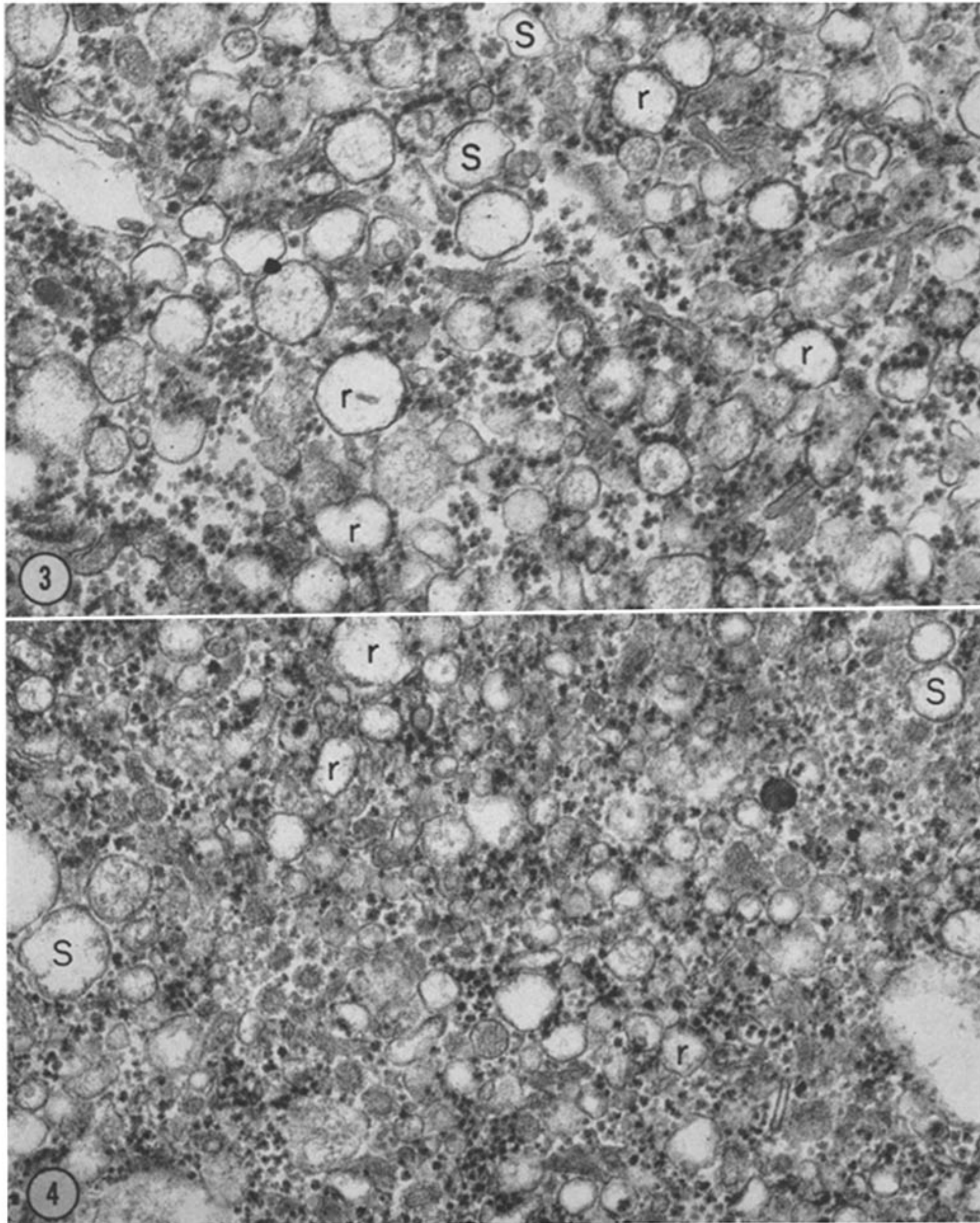
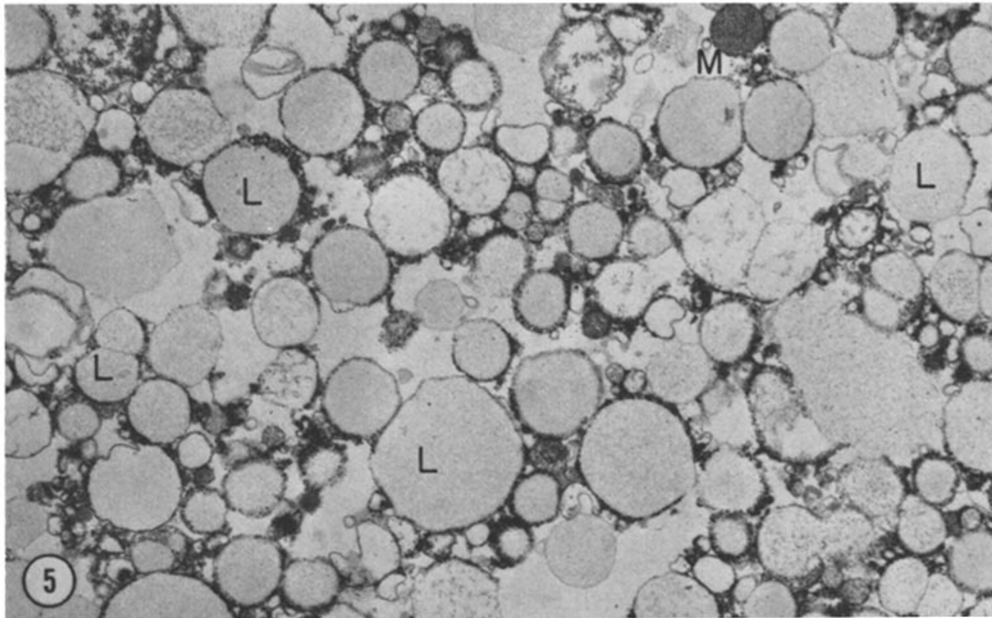


FIGURE 3 Microsomal pellet (liver from normal rat), illustrating numerous small vesicles of both rough (r) and smooth (s) endoplasmic reticulum, membranes, and free particles. The majority of the latter are glycogen. The majority of ribosomes are membrane-bounded, free ribosomes are rare. The ratio of rough to smooth vesicles is approximately 3. × 40,000.

FIGURE 4 Microsomal pellet (liver from orotic acid-fed rat), illustrating vesicles of rough (r) and smooth (s) endoplasmic reticulum, membranes, and free particles. As in the control, most of the free particles are glycogen and most of the ribosomes that are visible are membrane-bounded. The ratio of rough to smooth vesicles is 1, indicating a considerable relative decrease in rough endoplasmic reticulum. × 40,000.



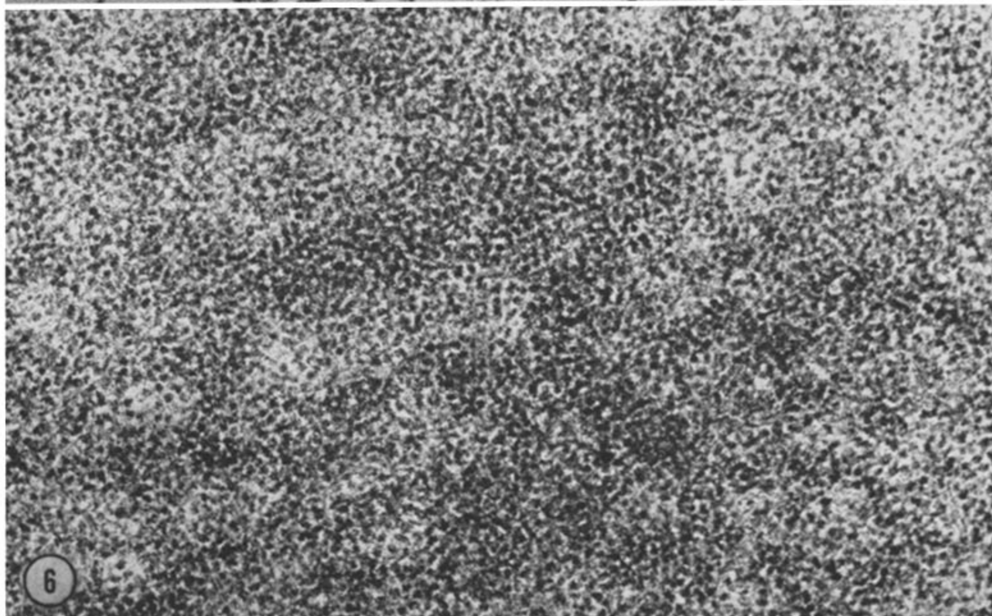


FIGURE 5 Washed low density structures prepared from the liver of a rat fed the orotic acid diet. The membrane-bounded vesicles contain material that is faintly osmiophilic (L) and presumably contains a high proportion of neutral lipid (triglycerides). The vesicles resemble and are presumably identical with the endoplasmic reticulum-derived cytoplasmic vacuoles (L in Fig. 2). Despite careful washing and controlled centrifugation, a rare mitochondrion (M) was present among the low density structures. × 10,000.

FIGURE 6 Ribosomal pellet prepared by treatment of the low density structures (shown in Fig. 5) with 0.5% deoxycholate. The preparation is homogeneous and contains only a few membranes. In size and morphology these ribosomal particles do not differ from similar particles prepared from normal rat liver. × 60,000.

material. The amount of this contamination was reduced by suspending the original pellicle in the sucrose-homogenizing medium and recentrifuging The sediment from this centrifugation was particularly rich in mitochondria. When the low density structures were treated with deoxycholate (0.5%) and centrifuged, a clear oil with no distinguishable structure was observed at the top of the tube. The sediment from this preparation was composed primarily of ribosomal particles with minor amounts of membranous material (Fig. 6). The supernatant fraction from the deoxycholate treatment contained insignificant amounts of RNA. It is concluded, therefore, that the RNA in the low density structures is ribosomal and not a membrane-bounded fraction. The average dimensions of these ribosomal particles were the same as those of ribosomes isolated from the microsomal fraction of normal liver. On the basis of these findings, the method of preparation, and the relative abundance of these particles, it is concluded that these particles are not the smaller ribosomes isolated from mitochondria (29).

Centrifugation of a homogenate from control rats yielded a material that was similar in gross appearance to the low density structures, but the quantity was so small that no estimation of protein or nucleic acid was possible. In the experiment in which control and fatty livers were homogenized together, evidence was obtained for the occurrence of some entrainment of particulate material from the control livers. However, the specific activity of the RNA of the low density structures isolated from the mixture of liver from a control rat previously given 8-^{14}C-adenine and unlabeled liver from the orotic acid–treated rat indicated that only 25% of the RNA found in the low density structures was derived from the control liver (Table I). This radioactive RNA may be attributable to the negligible quantity of floating material from the control rat and would have, therefore, appeared along with the low density structures. Alternatively, it may simply be attributable to nonspecific physical occlusion by the lipid present in the latter structures. Similarly, when the pellicle of low density structures was prepared after labeling the liver of the orotic acid–treated rat instead of the control rat, the specific radioactivity was diluted by 22%. These results indicate that the major portion of the RNA present in the low density fraction is derived from intrinsic low density structures in the orotic acid–induced fatty livers and not from entrainment of cytoplasmic components.

The composition of the various elements isolated from the livers of rats treated with orotic acid is presented in Table II. The ratio of protein to RNA in the low density structures in nearly three times greater than that found in the microsomal fraction of the livers from the control or orotic acid–treated rats. The ratio of protein to RNA in the ribosomes from the low density structures after deoxycholate treatment, however, was 1.0, a value identical with that found for the ribosomes derived from the microsomal fraction from the livers of both the control and the orotic acid–treated rat.

TABLE I

Occlusion of Fragments from Normal Liver in the Pellicle from a Homogenate of Orotic Acid–Induced Fatty Liver

Experiment	Liver labeled	Sample analyzed	Specific activity	Dilution of the specific activity
			cpm/mg RNA	%
1	Control liver	Microsomal RNA of the labeled liver	8,067	—
		RNA from low density structures	2,000	75
2	Orotic acid-induced fatty liver	Microsomal RNA of the labeled liver	26,217	—
		RNA from low density structures	19,571	22

10 μc of ^{14}C-adenine was administered intraperitoneally 24 hr before killing. Equal weights of the livers were homogenized together and the specific radioactivity of the RNA was determined as described in Materials and Methods.

TABLE II

Protein and RNA Composition of the Microsomes and the Low Density Structures from Orotic Acid–Induced Fatty Livers

Samples analyzed	Protein	RNA	Ratio	
			Before deoxycholate treatment	After deoxycholate treatment (ribosome pellet)
	mg/g wet liver ± SE		*mg protein/mg RNA*	
Microsomes from control rat liver	18.8 ± 0.75 (3)	2.64 ± 0.18 (3)	7.10	1.0
Microsomes from orotic acid–induced fatty liver	17.5 ± 1.40 (3)	2.70 ± 0.34 (3)	6.50	1.0
Low density structures	5.5 ± 0.70 (6)	0.26 ± 0.03 (6)	25.00	1.0

The isolation of microsomes, low density structures, ribosomes, and the estimation of RNA are described under Materials and Methods. Protein was estimated by the method of Lowry et al. (41). The values in parentheses represent the number of samples analyzed.

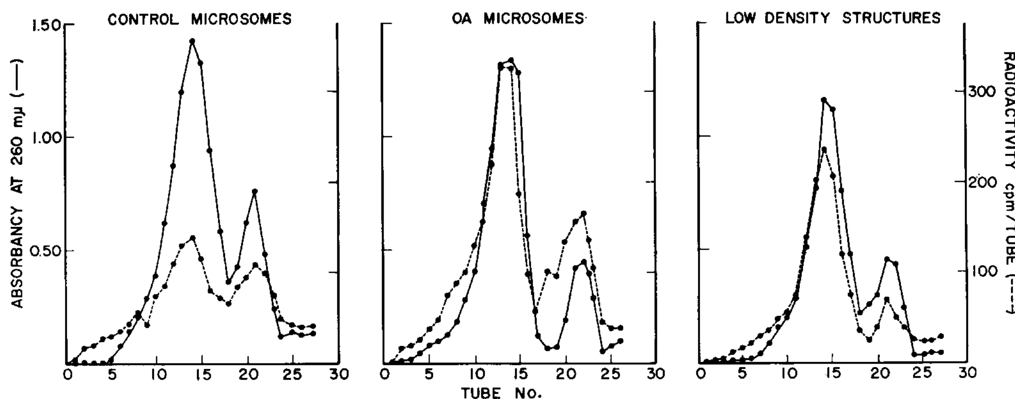


FIGURE 7 Sedimentation pattern of ribosomal RNA from the livers of control and orotic acid-fed rats. Each rat was given 30 μc of 8-^{14}C-adenine intraperitoneally 1 hr before sacrifice. RNA (about 6 $A_{260\ m\mu}$ units) was layered on 50 ml of a linear sucrose gradient (5–20%) containing 0.1 N NaCl and 0.01 N sodium acetate (pH 5.1) and centrifuged for 15 hr at 20,000 rpm at 4°C in a 25.2 rotor in a Spinco model L2-65. The solid line represents $A_{260\ m\mu}$ and the dashed line represents radioactivity.

The sedimentation profiles of the RNA isolated from the ribosomes derived from the microsomes of the normal liver and the orotic acid–induced fatty livers are represented in Fig. 7. The two peaks seen in Fig. 7 correspond to the 29S and 18S RNA of ribosomal RNA; RNA isolated from the soluble fraction of the liver appeared after the second peak. The relative position of the absorbancy at 260 mμ and the radioactivity in the sedimentation profile obtained from the RNA associated with the low density structures is very similar to that of ribosomal RNA. The specific activity values of the 29S and 18S RNA fractions 1 hr after the injection of 8-^{14}C-adenine, however, are very different (Table III). In the ribosomes isolated from the microsomal fraction of the liver from control and orotic acid–treated animals, the specific radioactivity of the 18S RNA is about 2-fold greater than that of the 29S RNA, in confirmation of earlier studies (30). In the RNA isolated from the ribosomes found in the low density structures, however, the specific radioactivity of the 29S RNA is greater than or equal to that of the 18S RNA. It is also interesting to note that the specific radioactivities of both the 29S and 18S RNA of the low density structures are lower than that of the corresponding

TABLE III

Specific Radioactivity of Ribosomal RNA after Fractionation on Sucrose Density Gradient Centrifugation

RNA	Sources of ribosomal RNA: Control liver microsomes	Orotic acid–induced liver microsomes	Low density structures
29S, cmp/$A_{260\ m\mu}$	80	464	393
18S, "	193	791	282
Ratio, 18S/29S	2.03 ± 0.23	1.36 ± 0.38	0.96 ± 0.12

Each rat received 30 μc of 8-^{14}C-adenine intraperitoneally 1 hr before killing. The experimental details are given in Materials and Methods. The ratio values represent the average of three different experiments, with the standard error.

TABLE IV

Base Composition of the RNA from Low Density Structures

Samples analyzed	Adenylic acid	Guanylic acid	Uridylic acid	Cytidylic acid
		μmoles/100 μmoles of nucleotides ± SE		
Microsomal RNA of control rat liver	18.4 ± 0.42	34.3 ± 0.38	16.2 ± 0.46	31.2 ± 0.60
RNA from the low density structures	19.3 ± 0.28	38.1 + 0.71	17.9 ± 0.70	25.1 ± 0.59

The analyses represent the average of four experiments and were performed as described in Materials and Methods.

ribosomal RNA from the microsomal fraction of the orotic acid–treated rat. This is surprising if one assumes that both populations of ribosomes are derived from the same endoplasmic reticulum. The higher specific radioactivity of both the RNA of the low density structures and the RNA of the microsomal ribosomes of the orotic acid–treated rat, compared to the ribosomal RNA of the control rat, is probably attributable to the reduction in the pool of adenine nucleotides in the animals fed the orotic acid diet (9).

The base composition of the RNA of the low density structures (Table IV) is different from that of microsomal RNA. The RNA of the low density structures has a relatively greater amount of guanylic acid and a reduction in the amount of cytidylic acid when compared to normal microsomal RNA.

DISCUSSION

The appearance of lipid within the endoplasmic reticulum of the hepatic cell has been noticed after administration of compounds which induce fatty livers (16–21) and also during the early stages of liver regeneration (31, 32). In the present studies structures similar to endoplasmic reticulum in association with large quantities of lipid have been isolated by centrifugation. The biochemical findings on these structures, particularly the release of ribosomes by deoxycholate, strongly suggest the presence of some elements of rough endoplasmic reticulum as well as smooth endoplasmic reticulum in this material. It is interesting to note that these "floated microsomes" contain a greater proportion of deoxycholate-soluble protein relative to RNA than the microsomes isolated by sedimentation from the same liver. Although this may reflect contamination with other cellular elements, the increased content of lipoprotein in this fraction is consistent with the presence of considerable amounts of smooth endoplasmic reticulum. Alternatively, high levels of lipoprotein may reflect an impairment of the secretion of this material (22).

The pattern of labeling of the 29S and 18S RNA of the ribosomes derived from the low density structures is very similar to that reported by Bergeron-Bouvet and Moulé (30) for the RNA isolated from the membranes of endoplasmic reticu-

lum. Since the ribosomes of the low density structures constitute only a small portion of the total cellular pool of ribosomes, the RNA associated with these structures may represent a distinctly different population of RNA. The analysis of base composition of the RNA isolated from the low density structures suggested a difference between this RNA and the total microsomal RNA. The base ratio of this RNA from low density structures is similar to that reported for the RNA associated with the membrane of the endoplasmic reticulum by Chauveau et al. (33). Nevertheless, caution must be used in the interpretation of the base composition because the sensitivity of different species of RNA to endogenous ribonuclease during isolation varies greatly. Others have shown, however, that tissues engaged in an active secretion of protein, such as the pancreas and lactating mammary glands, contain RNA rich in guanylic acid (34, 35).

The sequence of biochemical events leading to the formation of lipoproteins and their secretion is uncertain. It is known, however, that esterification of fatty acids and synthesis of serum lipoproteins occur in the endoplasmic reticulum of the hepatic cell (36–38). It seems possible that after lipids are formed they are released into the cisternae of the endoplasmic reticulum. At the same or different sites in the reticulum, the apolipoprotein and the lipid are assembled into the completed lipoprotein and secreted into the plasma. In this hypothetical sequence of events, a specific block after the formation of the protein moiety of the lipoprotein, as seen in orotic acid–induced fatty livers, would lead to an accumulation of lipid in the rough endoplasmic reticulum. Homogenization of these livers would result in the fragmentation of the endoplasmic reticulum, and those portions that had accumulated lipid *in situ* would tend to float instead of sedimenting during centrifugation; this material would appear as a pellicle similar to the low density structures reported in the current work.

Compounds such as CCl_4, ethionine, azaserine, 4-amino-pyrazolopyrimidine, and orotic acid which induce fatty liver also create a deficiency of ATP in the liver (9, 15, 39, 40). Since all these compounds except orotic acid have been shown to inhibit protein synthesis, their primary effect could be assumed to be on the synthesis of lipoprotein rather than its secretion. The available evidence indicates that orotic acid does not affect protein synthesis (22–24). Furthermore, the work of Roheim et al. suggests that the lipoprotein is not assembled or secreted until after the protein moiety of the lipoprotein is made (22). This unique feature of the effect orotic acid implicates the role of purine or pyrimidine nucleotides in the secretion of lipoproteins. It is generally believed that the secretion of lipoproteins is one of the selective functions of the membrane of the endoplasmic reticulum. If this is an energy-requiring phenomenon and is dependent upon a critical concentration of ATP, it is possible that a reduction in the intracellular concentration of ATP as seen in orotic acid–fed rats would interfere with this secretory function of the cell.

After the completion of this work, a paper appeared describing the isolation of structures (liposomes) from the liver of rats treated with ethionine that are very similar to the structures reported in this paper (42). Evidence for the derivation of liposomes from both rough and smooth elements of the endoplasmic reticulum was presented; these liposomes were found to have a protein:RNA ratio similar to that of the low density structures in this report.

The structures demonstrated in this report may be a normal portion of the endoplasmic reticulum which is fractionated from the majority of the reticulum in the cell by their engorgement with lipid, or they may constitute a pathologically modified segment of the normal reticulum. The present data do not allow selection between these two possibilities. In either case, the base composition of RNA from the low density structures and the isotopic labeling of their ribosomes lend support to the evidence reported by others (43, 44) that the total population of ribosomes within the liver cell may not be homogeneous. Alternatively, some of the ribosomes may undergo modification when the animal is fed a diet containing orotic acid.

This work has been supported in part by a grant (T112F) from the American Cancer Society, a fellowship from the Jane Coffin Childs Memorial Fund of Medical Research, and a research grant (AI-06584) from the National Institute of Allergy and Infectious Diseases of the United States Public Health Service. Support of R. E. Handschumacher as an American Cancer Society Professor is gratefully acknowledged. Miss L. Wallmark, Mrs. P. Wong, and Mrs. G. Jost have given expert technical assistance in this work.

Received for publication 27 December 1967, and in revised form 9 January 1969.

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