LECITHIN SYNTHESIS, INTRACELLULAR TRANSPORT, AND SECRETION IN RAT LIVER

IV. A Radioautographic and Biochemical Study of Choline-Deficient Rats Injected with Choline-^{3}H

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ABSTRACT

Injection of choline-^{3}H into choline-deficient rats resulted in an enhanced incorporation of the label into liver lecithin, as compared to the incorporation of label into liver lecithin of normal rats. The results obtained with the use of different lecithin precursors indicate that in the intact liver cell, both in vivo and in vitro, exchange of choline with phosphatidylcholine is not significant. The synthesis and secretion of lecithins by the choline-deficient liver compare favorably with the liver of choline-supplemented rats, when both are presented with labeled choline or lysolecithin as lecithin precursors. Radioautography of the choline-deficient liver shows that 5 min after injection of choline-^{3}H the newly synthesized lecithin is found in the endoplasmic reticulum (62%), mitochondria (13%), and at the "cell boundary" (20%). The ratio of the specific activity of microsomal and mitochondrial lecithin, labeled with choline, glycerol, or linoleate, was 1.53 at 5 min after injection, but the ratio of the specific activity of phosphatidyl ethanolamine (PE), labeled with ethanolamine, was 5.3. These results indicate that lecithin and PE are synthesized mainly in the endoplasmic reticulum, and are transferred into mitochondria at different rates. The site of a precursor pool of bile lecithin was studied in the intact rat and in the perfused liver. Following labeling with choline-^{3}H, microsomal lecithin isolated from perfused liver had a specific activity lower than that of bile lecithin, but the specific activity of microsomal linoleyl lecithin was comparable to that of bile lecithin between 30 and 90 min of perfusion. It is proposed that the site of the bile lecithin pool is located in the endoplasmic reticulum and that the pool consists mostly of linoleyl lecithin.

INTRODUCTION

The concept that membranes are elementary structures extensively used in the construction of subcellular components and that phospholipids are an integral part of all membranes, raises the question as to the site of synthesis of membrane phospholipids. The enzymes involved in the formation of lecithin and phosphatidyl ethanolamine have been localized to the microsomes (1), which are considered the predominant site of lipid synthesis (2, 3).

This fraction might be envisaged as the source of phospholipids destined to become both struc-

tural components of membranes and parts of secretory products, i.e. bile and plasma phospholipids. This concept of unilocular phospholipid synthesis has been recently questioned, especially with respect to mitochondrial and biliary lecithin. Since 10 min after injection of labeled choline, the ratio of the specific activity of microsomal to mitochondrial lecithin was about 2.0, the possibility of independent mitochondrial synthesis of lecithin remained open (4).

Similarly, the source of biliary lecithin has not been localized so far. Zilversmit and Van Handel (5) have found that after ^{32}P injection the lecithin in the bile attains a specific activity higher than that of the whole liver lecithin. They postulated, therefore, that biliary lecithin might be derived from a special compartment in the liver. As the specific activity of bile lecithin exceeded that of microsomal lecithin following injection of several lecithin precursors (6), the question whether the microsomes can be identified with the site of the bile lecithin compartment was not answered conclusively.

The present report is an attempt to find out the origin of mitochondrial lecithin and the localization of the precursor pool of biliary lecithin in the intact cell, with the help of electron microscopic radioautography. For this purpose, choline seemed to be the most specific marker of lecithin, but for the possibility that it might be incorporated through an exchange reaction (7), and might bypass the accepted pathway of lecithin formation (8). Hence, in the present study the occurrence of such an exchange reaction in the intact liver cell was evaluated.

As the amount of label present in the specimen limits the feasibility of radioautography and as the uptake of labeled choline is quite low in the normal animal, use was made of choline-deficient rats. This report includes also a comparative study of lecithin synthesis and secretion in choline-deficient and choline-supplemented rats.

MATERIALS AND METHODS

Animals

Male albino rats of the Sprague-Dawley strain were used. The rats were fed Purina laboratory chow or a synthetic diet deficient in choline (Nutritional Biochemical Corporation, Cleveland), or the same diet, supplemented with choline chloride, 0.6 g/kg.

Subcellular Fractionation

Following intravenous injection of labeled tracers, the rats were killed by exsanguination under light ether anesthesia, and the livers removed and chilled in ice-cold 0.25 M sucrose. A 10% homogenate was prepared in 0.25 M sucrose in a Potter-Elvejhem homogenizer with a teflon pestle. Nuclei and debris were removed by centrifugation at 1000 *g* max for 10 min. Mitochondria were isolated at 10,000 *g* av for 15 min and, following rehomogenization in the centrifuge tube and resuspension in the same volume of 0.25 M sucrose, they were recentrifuged at 6600 *g* av for 10 min. Microsomes were sedimented at 100,000 *g* av for 120 min. The fractions were checked for purity by electron microscopy. For determining the admixture of microsomes in the mitochondrial fraction, liver homogenates were prepared from two rats, one of which was injected with choline-^3H. After removal of nuclei and debris, mitochondria were sedimented at 10,000 *g* av for 15 min. The pellet derived from the unlabeled homogenate was then resuspended in the labeled supernate and reisolated as described above. The radioactivity recovered in the washed mitochondrial pellet was taken as an estimate of microsomal contamination, which ranged between 4 and 5%.

Liver Slices

Liver slices 70–100 mg in weight were prepared with a Stadie-Riggs tissue slicer and collected in ice-cold Krebs-Ringer phosphate, pH 7.4. The slices were incubated in the same medium containing 5 mM glucose and the radioactive choline, under constant oxygenation, in a shaking incubator at 37°. In experiments of the pulse-chase type, following labeling with choline-^3H or ethanolamine-^3H, either in vitro or by injection in vivo, the slices were transferred to 15 ml of the medium containing 500 μg/ml of either cold choline or ethanolamine, respectively. At the end of the experiment the slices were blotted, weighed, and one portion was homogenized in chloroform:methanol, 2:1 (v/v), the other was fixed for electron microscopy.

Cannulation of the Bile Duct

A Pe 10 polyethylene tube was tied into the proximal third of the bile duct, threaded through a subcutaneous tunnel, and exteriorized in the interscapular region. The rats were placed in restraining cages and were given food and 0.5% sodium chloride–0.2% potassium chloride ad lib. Bile was collected overnight in ice. Rats with a consistent bile flow were used on the morning following the operation.

Liver Perfusion

Liver perfusion was carried out as described by Miller et al. (9). Labeling by recirculation with 5–10 mc of choline-^{3}H was carried out before the introduction of the liver into the perfusion chamber. The labeling solution, 50 ml, consisted of rat red blood cells suspended in 0.9% NaCl with a hematocrit of 30%. After 5–7 min of labeling, 50 ml of nonlabeled perfusion medium were passed through the liver in order to wash out the radioactivity from the vascular compartment. The liver was then introduced into the chamber, and recirculation perfusion was continued for 120 min. The perfusion medium consisted of rat red blood cells resuspended in rat serum with a hematocrit of 30%, and contained 1 mg/ml of choline chloride. The ratio of pulse to chase choline was 1:1000, and the total volume of the perfusate was 150 ml.

Analytical and Chromatographic Procedures

Lipids were extracted with chloroform:methanol, 2:1 (v/v), according to Folch et al. (10). The chloroform was washed three times with the reconstituted pure solvent upper phase in order to remove the last traces of the labeled water-soluble precursors and their metabolites. The total lipids were first adsorbed on silicic acid columns (11) and neutral lipids were eluted with chloroform and phospholipids were eluted with methanol, containing 3% of water. Alternately, they were directly fractionated by thin layer chromatography (Camag, Kieselgel, type DO, 0.5 mm thick) according to Skipski et al. (12), with the use of a solvent system of chloroform:methanol:acetic acid:water (50:25:9:4). This system gave a good separation between lecithin and phosphatidyl inositol and between phosphatidyl ethanolamine and serine. The fractions identified with the help of reference standards were scraped off the plate into counting vials containing 0.5 ml of methanol. Scintillation fluid (13) was added, and the samples were counted in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Downer's Grove, Ill.). In experiments in which the specific activity of a given phospholipid fraction was determined, the fraction to be examined was extracted according to Goldrick and Hirsch (14), with two times 10 ml of methanol containing 3% of water, and aliquots were taken for specific activity and phosphorus determination. In some experiments, lecithin was further separated into families on silver nitrate-impregnated silica gel plates with a solvent system consisting of chloroform:methanol:water, 60:25:3 (v/v) (15). This separation resulted in three fractions: Fraction I had an Rf of 0.45 and its predominant unsaturated fatty acids were linoleic acid and oleic acid; Fraction II had virtually no linoleic acid and contained most of the arachidonic acid of the original lecithin; Fraction III which remained close to the origin contained the more polyunsaturated fatty acids. The various lecithin fractions were eluted from cation exchange resin columns with methanol:acetic acid:water, 94:1:5 (v/v), as described by Kyriakides and Balint (15). The fatty acids were methylated and separated by gas-liquid chromatography (16). Lipid phosphorus was determined according to Bartlett (17) and, in samples containing little phosphorus, according to Chen et al. (18). Triglycerides were estimated as described by Hatch and Lees (19). All organic solvents were glass distilled.

Labeled Precursors

Choline (Me-^{3}H) chloride, specific activity 6.7 c/mmole, ethanolamine-2-^{3}H hydrochloride, specific activity 1.94 c/mmole, were obtained from Radiochemical Center, Amersham, England. Linoleic acid-1-^{14}C, glycerol-2-^{3}H, choline (Me-^{14}C) chloride, choline-1-2-^{14}C chloride, and ethanolamine-1,2-^{14}C hydrochloride were obtained from New England Nuclear Corporation, Boston. Rat liver lecithin was labeled biosynthetically with glycerol-2-^{3}H or with choline-1,2-^{14}C or choline-Me-^{3}H and was converted to lysolecithin (1-acyl-Sn-glycerol-3-phosphorylcholine) by the use of *Crotalus adamanteus* venom (20). Before intravenous injection, the lysolecithin was homogenized in 0.9% NaCl in an all-glass conical homogenizer and subjected to sonication for 3 min at 9 kc. Linoleic acid-1-^{14}C was converted to a sodium salt and complexed to rat serum as described before (21).

Preparation of Specimens for Electron Microscopy and Radioautography

Samples of liver were fixed in Karnovsky's fixative (22) for 2 hr and were washed with five changes of 0.1 M cacodylate buffer, the last change overnight at 4°. The tissues were postfixed in 2% osmium tetroxide in acetate Veronal buffer for 1–2 hr and were dehydrated at 0° in two changes of 70% ethanol, 5 min each, one change of 95% ethanol for 5 min, and three changes of Epon 812, 1 hr each. Infiltration was carried out overnight at 4° in a complete Epon mixture, with the accelerator. The tissue was embedded in Epon according to Luft (23). Sections prepared on a Porter-Blum Servall MII microtome with a diamond knife were collected on Formvar-coated grids. Radioautography was performed according to Caro and Van Tubergen, with the use of the Ilford L4 emulsion (24). Following exposure at 4° the grids were developed in Microdol X, (Eastman Kodak Co., Rochester, N. Y.), fixed in Kodak fixer, and, following brief washing in water, were stained with lead citrate (25, 26) and examined with a Siemens electron micro-

TABLE I

Elimination of Water-Soluble-^3H during Fixation and Washing after Labeling with Choline-^3H

Sample	Distribution of radioactivity in liver		
	Water phase	Chloroform phase	"Protein cake"
	%	%	%
Unfixed liver	80.7	19.0	0.3
Fixative* + wash	80.0	0	—
Fixed liver	0.7	18.5	0.8

* Karnovsky's formaldehyde-glutaraldehyde fixative; the 100% in the case of the fixed liver is obtained by adding the radioactivity recovered in the fixative + wash.

scope at 80 kv. Grain counts were performed as described previously (27); those grains which were partially over mitochondria were included in the mitochondrial count if two-thirds or more of the grain was located over the mitochondrion; otherwise, the grain was counted as label of endoplasmic reticulum.

The elimination of the water-soluble precursors, before embedding, and the loss of labeled lipid during dehydration were monitored by liquid-scintillation counting. As seen in Table I, fixation in Karnovsky's formaldehyde-glutaraldehyde fixative, followed by an overnight wash in 0.1 M cacodylate buffer, resulted in the elimination of most of the water-soluble radioactivity from liver labeled with choline-^3H. The remaining 0.7% radioactivity was eliminated in osmium tetroxide and 70% ethanol. The radioactivity found in the chloroform nonextractable residue (protein cake) was about 4% of the total radioactivity recovered in the fixed and washed tissue. In the case of ethanolamine-^3H, the elimination of the water-soluble precursors from the tissue was more difficult to evaluate, owing to a retention of the label in the protein cake when the tissue was homogenized in chloroform:methanol. 5–10 min after injection of ethanolamine-^3H, when the liver radioactivity in the water phase exceeded that in the chloroform phase, the label in the protein cake amounted to 16%. When the partition of radioactivity in the liver was in favor of the chloroform phase, which occurred 30–120 min after the injection of the label, or after 30–120 min of in vitro chase, not more than 1–2% of the label was found in the protein cake. Fixation in Karnovsky's fluid enhanced the retention of the label in the protein cake. Direct fixation in osmium tetroxide, which might not have enhanced the retention of label, was not used, owing to the difficulty of evaluating the efficiency of washout of the water-soluble precursor from osmium-tetroxide-fixed tissues. Owing to this complicating factor, radioautography was performed only on liver labeled for 10 min in vivo and chased in vitro for 30–120 min and on samples of liver obtained 1–2 hr after injection of ethanolamine-^3H. Table II shows that the loss of lipid radioactivity during sample preparation was minimal, as already reported previously when choline-^3H was used as label (28).

TABLE II

Preservation of Lipid Radioactivity during Preparation of Liver Samples for Electron Microscopy

	After injection with	
	Choline-^3H	Ethanolamine-^3H
	%	%
Osmium tetroxide	0.2	0.2
Ethanol 70%	0.7	0.3
Ethanol 95%	1.8	0.8
Epon 812	1.2	0.8
Epon mixture	0.3	0.2
Tissue	81.4	86.0

The lipid radioactivity recovered in the chloroform: methanol (2:1) extract of unfixed liver, labeled with choline-^3H or ethanolamine-^3H, was taken as 100%.

RESULTS

Specific Activity of Microsomal and Mitochondrial Lecithin and PE

For study of the origin of mitochondrial lecithin in the liver, normal rats weighing 150–200 g were injected with different precursors of lecithin, and subcellular fractions were prepared 5–120 min after injection. The precursors injected were: choline-^3H (3.5 mμmoles), linoleic acid-1-^{14}C (0.2 μmoles), glycerol-2-^3H (2 μmoles) and biosynthetically prepared lysolecithin, labeled with choline-^{14}C (1 μmole). As seen in Table III, when choline-^3H was used as precursor the ratio of the specific activity of lecithin, isolated from liver microsomes and mitochondria, at the earliest time studied, averaged 1.59. This ratio did not change appreciably during the next 60 min. A similar ratio of specific activity of lecithin in microsomes and mitochondria was found after injection of labeled glycerol or linoleic acid, and this ratio changed only little with time after injection. A higher ratio of specific activity of microsomal to mitochondrial lecithin was found 5 and 15 min after injection of labeled lysolecithin, and the ratio

TABLE III

Specific Activity of Microsomal and Mitochondrial Lecithin and Phosphatidyl Ethanolamine

Experiment	Injected labeled compound	Min	Specific activity: Microsomal LE or PE		Mitochondrial LE or PE		
			5	15	30	60	120
1	Choline-^{3}H		1.80	—	1.85	1.71	—
2	"		1.66	—	1.58	1.58	1.40
3*	"		1.50	—	—	1.60	1.20
4‡	"		1.40	—	1.70	1.50	1.10
5	Linoleic acid-^{14}C		1.48	1.49	1.64	—	—
5	Glycerol-^{3}H		1.37	1.43	1.68	—	—
6	Lysolecithin-^{14}C		2.16	2.26	1.66	—	—
7	Ethanolamine-^{3}H		5.05	3.49	2.87	—	—
8	"		5.65	—	4.90	2.89	2.20
3*	Ethanolamine-^{14}C		5.40	—	—	3.70	2.20

Specific activity of PE was measured only after injection of labeled ethanolamine and is shown in the last three lines.

In all experiments but experiment 4 each time point was derived from one normal rat.

* In experiment 3, the rats were injected with both choline-^{3}H and ethanolamine-^{14}C.

‡ In experiment 4, samples were obtained from the liver of a 4-day choline-deficient rat during perfusion in vitro.

approached that found with the other precursors 30 min after injection. The other major phospholipid component of mitochondria and microsomes, PE, was studied after injection of 5 mμmoles of ethanolamine-^{3}H. The ratio of specific activity of PE[1] from the two subcellular particulate fractions was much higher at the early time intervals and decreased slowly thereafter. In experiment 3, the subcellular fractions for each time interval were derived from rats injected with both choline-^{3}H and ethanolamine-^{14}C, and the same difference in the ratio was observed.

Uptake of Choline-^{3}H and Ethanolamine-^{3}H by Choline-Deficient and Normal Liver

The study of the interrelation between the mitochondrial and microsomal phospholipids was attempted in the intact cell with the help of radioautography. To that end specific labels for lecithin and PE were mandatory, and the feasibility of a pulse- and chase-type of experiment was explored. Since choline and ethanolamine are the only specific labels for lecithin and PE, respectively, the extent of their uptake by the liver was studied in rats 100–140 g in body weight. As seen in Table IV, the incorporation of choline into the phospholipids of normal rat liver is rather low and is not changed by feeding the choline-deficient diet for 1 day.

In rats kept on a choline-deficient diet for 2 days, the incorporation of choline into phospholipids was enhanced tenfold. Choline deficiency affected also the relative distribution of incorporated radioactivity between the chloroform and water-soluble fraction (the latter includes, in addition to choline, phosphorylcholine and CDP choline), with more label appearing in the chloroform phase. This finding was true for both tracer and large amounts of injected choline (Table IV). In the normal rat, and in the rat fed the choline-deficient diet for 1 day, the incorporation of choline into liver lipids increased with time up to 60 min after injection. In the 2- to 5-day choline-deficient rat, an extensive uptake of choline occurred at 5 min, and the increase with time was relatively lower both after a tracer dose and after a larger amount of choline. The uptake of labeled ethanolamine by the normal rat liver, when tracer doses or large doses were injected, is summarized in Table V. Choline proved to be a very specific label of lecithin, both in vivo and in liver slices, as at up to

[1] The following abbreviations were used; PE, phosphatidyl ethanolamine; LE, lecithin; SP, sphingomyelin; LL, lysolecithin; RER, rough endoplasmic reticulum; SER, smoooth endoplastic reticulum; CDP, cytidine diphosphate; CTP, cytidine triphosphate; ATP, adenosine triphosphate.

TABLE IV

Effect of Choline Deprivation on the Uptake of Choline-^3H by Rat Liver

Choline-deficient diet-fed	Choline-injected	Distribution of radioactivity in liver*			
		Chloroform phase		Aqueous phase	
		min 5	min 60	min 5	min 60
Days	*μmoles*	%	%	%	%
0	0.003	0.10	—	2.7	—
0	"	0.30	—	2.5	—
1	"	0.25	1.2	2.5	1.2
1	"	0.14	1.1	2.5	1.0
1	"	0.15	1.1	2.5	1.2
2	"	2.50	5.0	1.0	1.0
2	"	3.00	4.0	1.0	1.0
2	"	3.00	4.5	1.0	1.0
0	0.70	0.05	1.0	2.0	1.3
0	1.05	0.04	—	1.0	—
5	0.35	1.84	2.5	1.1	1.0
5	0.70	2.00	3.9	0.8	1.8

*Samples of liver obtained from the same rat 5 and 60 min after injection of choline-^3H were extracted in chloroform: methanol 2:1 and purified according to Folch et al. (10). The results are expressed as per cent of injected radioactivity per gram of wet weight of liver.

TABLE V

Uptake of Ethanolamine-^3H by Rat Liver

Ethanolamine-injected	Distribution of radioactivity in liver*			
	Chloroform phase		Water phase	
	min 10	min 120	min 10	min 120
μmoles	%	%	%	%
0.03	1.2	4.9	2.2	0.4
0.03	1.0	5.9	4.5	1.1
2.50	2.6	10.0	3.3	1.2
2.50	1.5	6.0	4.1	0.9

* Results are expressed as in Table IV.

120 min of incubation more than 98% of the phospholipid radioactivity was recovered in lecithin. Ethanolamine-^3H was found only in PE 10 min after injection and in slices chased in vitro for 120 min. In liver taken from the animal 2 hr after injection of the label, 90% of the lipid radioactivity was recovered in PE.

Evaluation of Choline Exchange

One objection to the use of choline was that, in liver homogenates, free choline has been reported

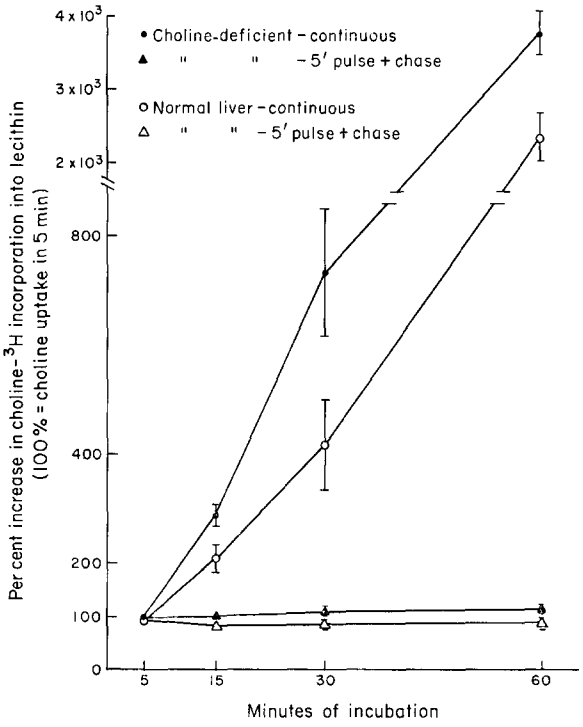


FIGURE 1 Liver slices of normal and 5-day choline-deficient rats were incubated for 5 min in 3 ml of medium containing 0.075 mM choline-^3H; a part of the slices was then post-incubated in 15 ml of medium containing 3.5 mM cold choline, or were labeled continuously for 60 min. There is no change in lipid radioactivity in the slices incubated in the presence of chase. Each point is a mean of four to five experiments ± SE.

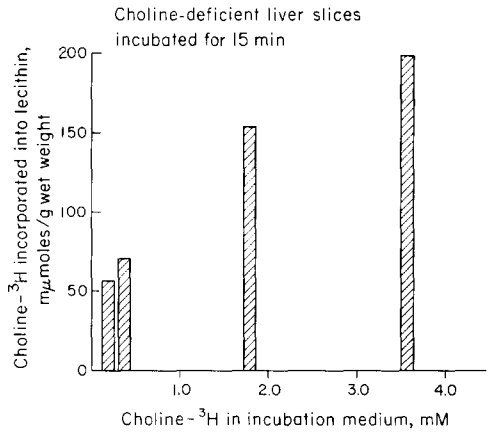


FIGURE 2 Slices of liver from 5-day choline-deficient rat incubated in the presence of high concentration of choline show good incorporation of the label into lecithin.

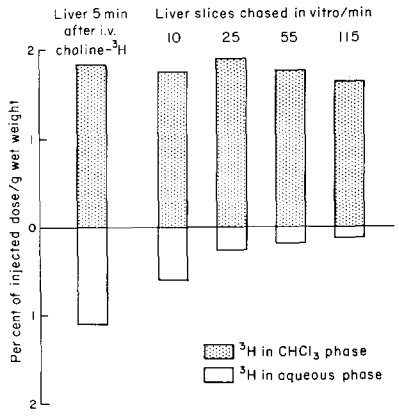


FIGURE 3 5 min after injection of 0.7 μmoles of choline-^{3}H into a choline-deficient rat, the liver was removed and slices were prepared and incubated in 15 ml of medium containing 3.5 mM cold choline. While the lipid radioactivity in the slice remains constant, there is a progressive elimination of water-soluble radioactivity.

to exchange with lecithin (7) and thus might not be a reliable marker of the sites of lecithin biosynthesis. Several experiments were devised as a test of whether such an exchange reaction takes place also in the intact cell. Liver slices were incubated with labeled choline for 5 min and then transferred to a medium containing an excess (1:250) of cold choline and incubated for 1–2 hr. As seen in Fig. 1, the amount of lipid radioactivity in the slices incubated with chase choline did not change with

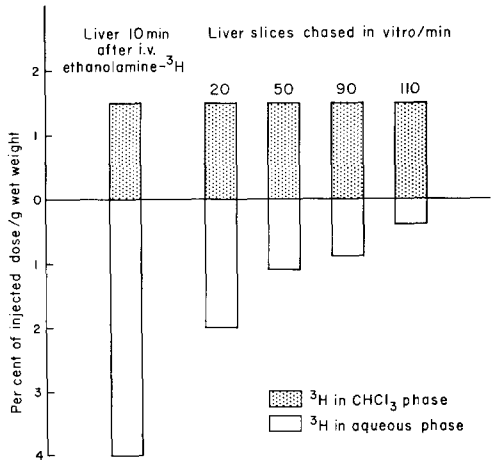


FIGURE 4 10 min after injection of 2.5 μmoles of ethanolamine-^{3}H into a normal rat, the liver was removed and liver slices were incubated in 15 ml of medium containing 5.0 mM of cold ethanolamine. While the lipid radioactivity in the slice remains constant, there is a progressive elimination of water-soluble radioactivity.

time, while a progressive increase in radioactivity was found in slices labeled continuously for 1 hr. As seen in Fig. 2, liver slices incubated in the presence of labeled choline, at concentrations used in the chase, showed a good incorporation of the label into lipids, a finding indicating that the concentration of choline was not deleterious. In another experiment, choline-deficient rats were injected with labeled choline, and slices, which were prepared from a lobe of the liver removed 5 min after injection, were incubated in the chase medium for up to 115 min. As in the previous experiment, the lipid radioactivity of the slices remained constant, while the water-soluble radioactivity decreased progressively (Fig. 3). Similar results were obtained also from liver slices with normal rats, labeled for 10 min in vivo with ethanolamine-^{3}H and chased in vitro for 2 hr (Fig. 4). These results did not seem to support the occurrence of the choline or ethanolamine exchange in the intact liver slice in vitro. The possibility of the choline-exchange reaction taking place in vivo was further investigated after injection of lysolecithin labeled in the glycerol with ^{3}H and in choline with ^{14}C. Since this precursor is taken up intact by the liver cell (29) and is rapidly converted to lecithin, the ratio of ^{3}H/^{14}C in the newly formed lecithin should be the same as in the injected lysolecithin.

TABLE VI

Preservation of $^{3}H/^{14}C$ Ratio in Liver Lecithin after Injection of Doubly-Labeled Lysolecithin (1-acyl-Sn-glycerol-^{3}H-3-phosphorylcholine-^{14}C)

	Ratio of $^{3}H/^{14}C$					
	Normal rats			Choline-deficient rats*		
	1	2	3	4	5	6
Injected lysolecithin	0.67	0.65	0.62	0.90	0.90	0.90
Liver lecithin	0.62	0.64	0.60	0.83	0.81	0.88
Serum lecithin	0.75	—	0.70	0.84	0.72	0.81

The labeled lysolecithin (1–2 μmoles) was injected i.v. and about 30% of the label was taken up by the liver in 120 min, of which 75% was converted to lecithin.

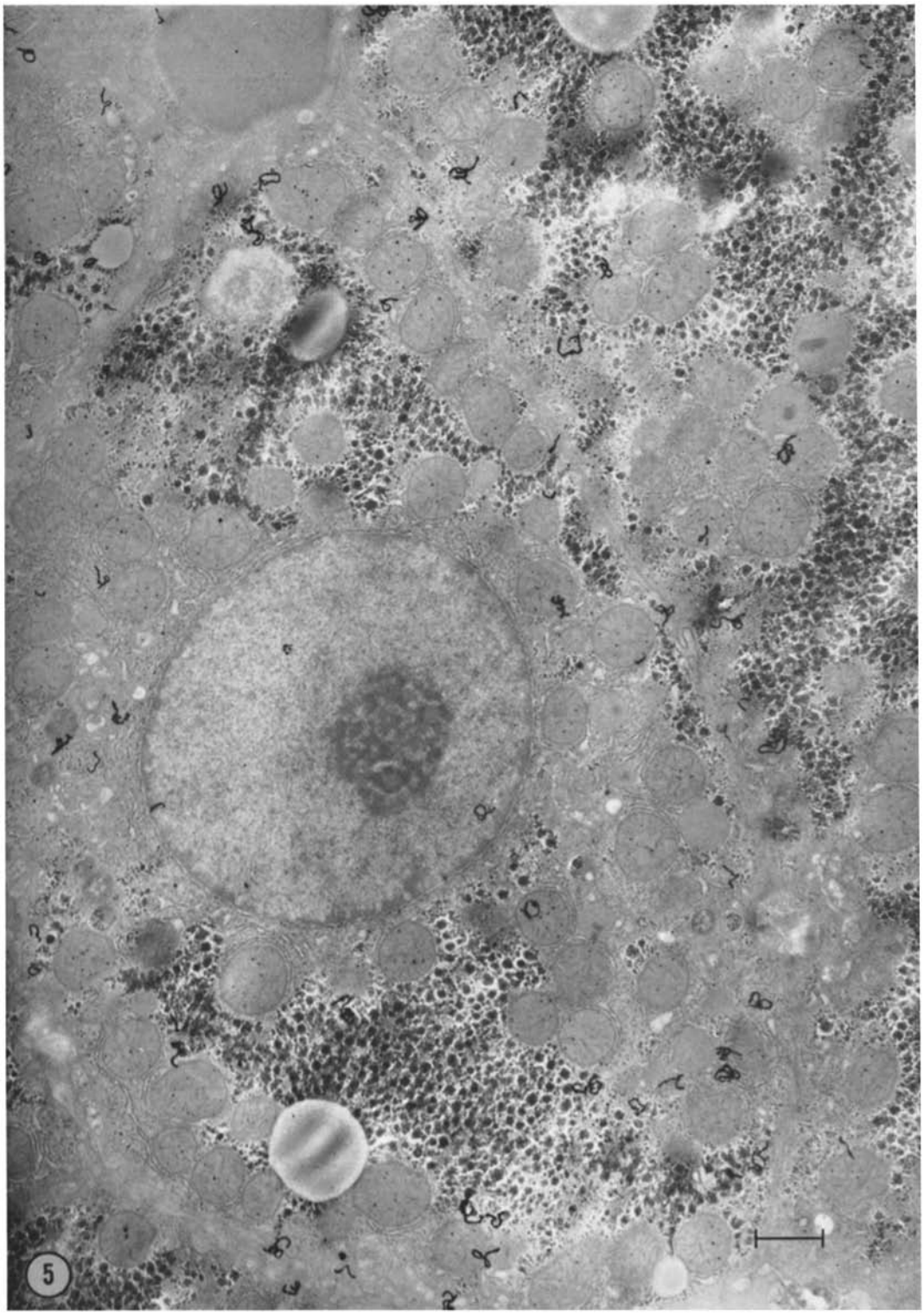
* The choline-deficient rats were injected with 5 mg of choline chloride intraperitoneally, immediately after injection of lysolecithin.

If free choline should exchange with the choline of phosphatidylcholine, the ratio of $^{3}H/^{14}C$ should rise markedly with time. However, as seen in Table VI the ratio of $^{3}H/^{14}C$ in the newly formed lecithin did not change appreciably for 2 hr, and the labeled lecithin secreted into the serum had a ratio of $^{3}H/^{14}C$, very close to that of the injected lysolecithin.

Radioautography

CHOLINE-^{3}H IN CHOLINE-DEFICIENT RATS: The liver of rats fed the choline-deficient diet for 4–5 days had a mean triglyceride content of 80 mg per g wet weight. In tissue sections, the lipid was seen in the form of multiple droplets of various sizes, mostly in the glycogen areas (30) (Figs. 5, 8, 12). There was also a greater accumulation of glycogen than in the liver of rats fed Purina. The endoplasmic reticulum, mitochondria, microbodies, and lysosomes did not differ from those seen in control liver. The Golgi cisternae were often dilated, and contained 50–80 mμ lipoprotein granules, which were less abundant than in normal liver, and were seen very rarely in the region ot the sinusoidal microvilli. Following in vitro incubation for 30–120 min, some of the slices showed a satisfactory preservation of ultrastructure (Figs. 10–12). There was no marked loss of glycogen or lipid during incubation, but at longer time intervals the smooth endoplasmic reticulum and the Golgi apparatus were less regular and somewhat dilated.

At 5 min after injection of choline-^{3}H, the radioautographic reaction was uniformly distributed throughout the cytoplasm of the cell (Figs. 5, 6). Grains were found associated with the various portions of the cell boundary (Figs. 5–7), and were seen over microvilli of the sinusoidal border (Figs. 5, 6) and over the microvilli of the bile canaliculi (Figs. 6, 7). Both the rough and smooth elements of the endoplasmic reticulum were labeled, and some grains were seen also over the Golgi apparatus, and over the nuclei where they were mainly close to the nuclear membrane (Figs. 5, 7). A considerable amount of label was associated with mitochondria, being 13% of the total grain count. Lysosomes and microbodies were labeled only occasionally. Usually, no grains were seen over the central portion of lipid droplets, but often they were quite numerous at the droplet boundary (Fig. 12). The distribution of the radioautographic reaction did not differ appreciably in samples of liver obtained 60 min after injection of choline, though somewhat more label was found over mitochondria, the Golgi apparatus, and the sinusoidal region (Figs. 8, 9) than at 5 min. Liver slices, prepared from the same lobe removed 5 min after injection and described above, were incubated in the presence of chase choline for an additional 115 min. The radioautographic reaction showed a distribution similar to that found in the 5-min samples as is shown in Figs. 10–12 and summarized in Table VII. The designation of cell boundary (Table VII) was chosen because the size and shape of the developed grains did not permit one to decide whether the grains were associated with the cell membrane proper, the cytoplasmic matrix of the microvilli, or any extracellular material. The same restriction must be applied also to any attempt at differentiation between structural and secretory lecithin, which is expected to be present at both secretory poles of the liver cell. Samples



FIGURES 5–12 Samples of liver of 5-day choline-deficient rats injected with choline-^{3}H.

FIGURES 5 and 6 5 min after injection. The radioautographic reaction is seen mostly over the elements of the endoplasmic reticulum, mitochondria, and "cell boundary" (comprising cell membrane, and microvilli of both the sinusoidal and biliary surface). The lipid droplets are not labeled. Fig. 5, × 10,000; Fig. 6, × 11,000.

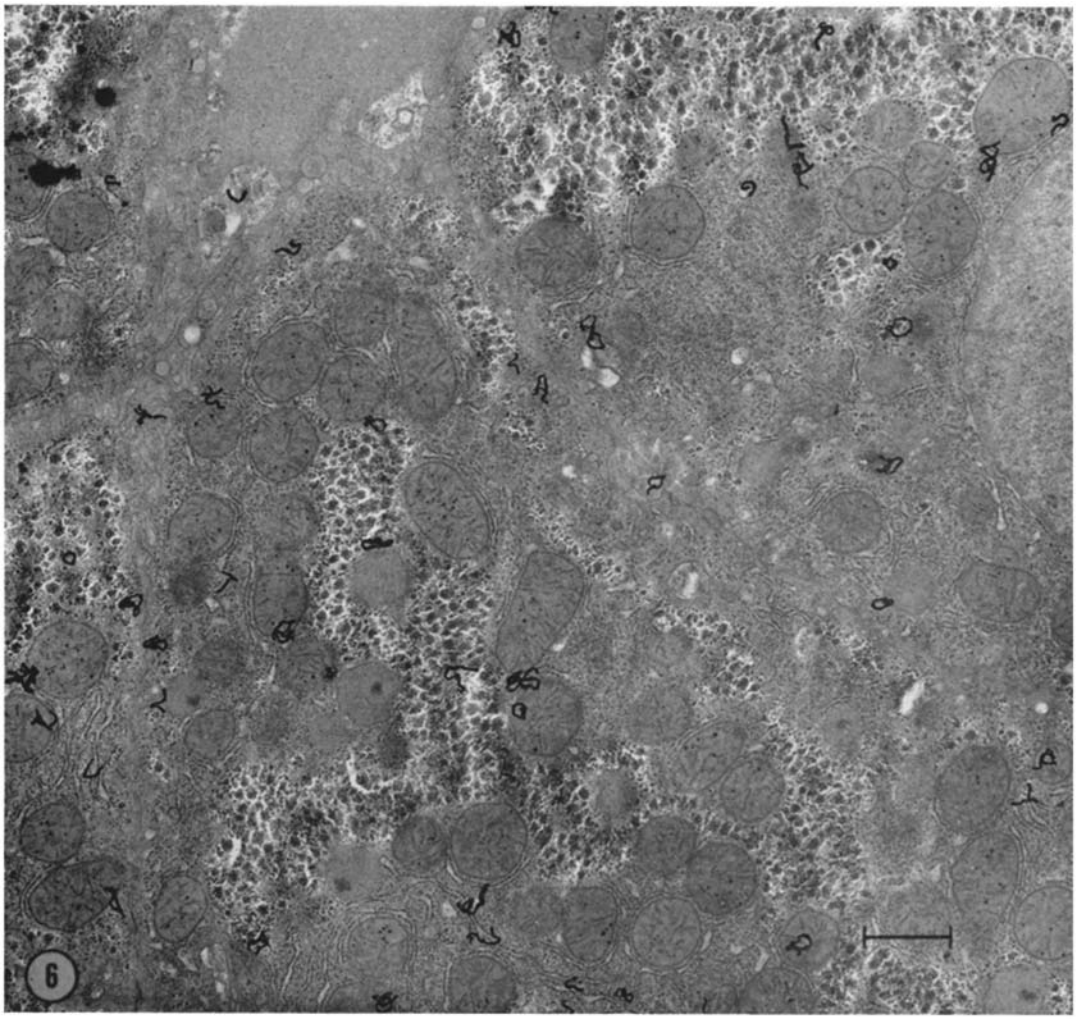


FIGURE 6 See legend under Fig. 5.

obtained from livers of choline-deficient rats, perfused in vitro for up to 2 hr, showed a satisfactory preservation of ultrastructure. The distribution of the radioautographic reaction was similar to that seen in the livers of intact rats injected with labeled choline.

ETHANOLAMINE-^{3}H IN NORMAL RATS: The liver samples used for radioautography were only those which contained relatively little water-soluble radioactivity, i.e. 30–120 min after injection of ethanolamine. At these time intervals, the distribution of the label, of which 90% was in PE, was uniform throughout the liver cell, and the grains were mostly associated with the elements of the endoplasmic reticulum and mitochondria. Fewer grains were seen in the region of the sinusoidal microvilli and over the bile capillary than after injection of choline-^{3}H at any time interval studied (Fig. 13). Grain counts performed on radioautographs of liver 120 min after injection showed the following per cent distribution: 37, RER; 26, SER; 21, mitochondria; 2, nuclei; 2, Golgi apparatus; 1, bile capillary; 3, lateral cell border; 8, sinusoidal cell border. Total number of grains counted was 400.

Bile and Serum Lecithin

The next series of experiments deals with the intracellular origin of biliary lecithin. Choline-

deficient and choline-supplemented rats were injected each with choline-^{14}C and lysolecithin-choline-^{3}H and the incorporation of both precursors into liver, bile, and serum lecithin was followed for 6 hr after injection. A possibility was envisaged that serum lysolecithin following acylation to lecithin might serve as a direct precursor of biliary lecithin and be secreted into the bile, without mixing with the liver lecithin pool. No marked difference in the 6-hr bile flow and in the

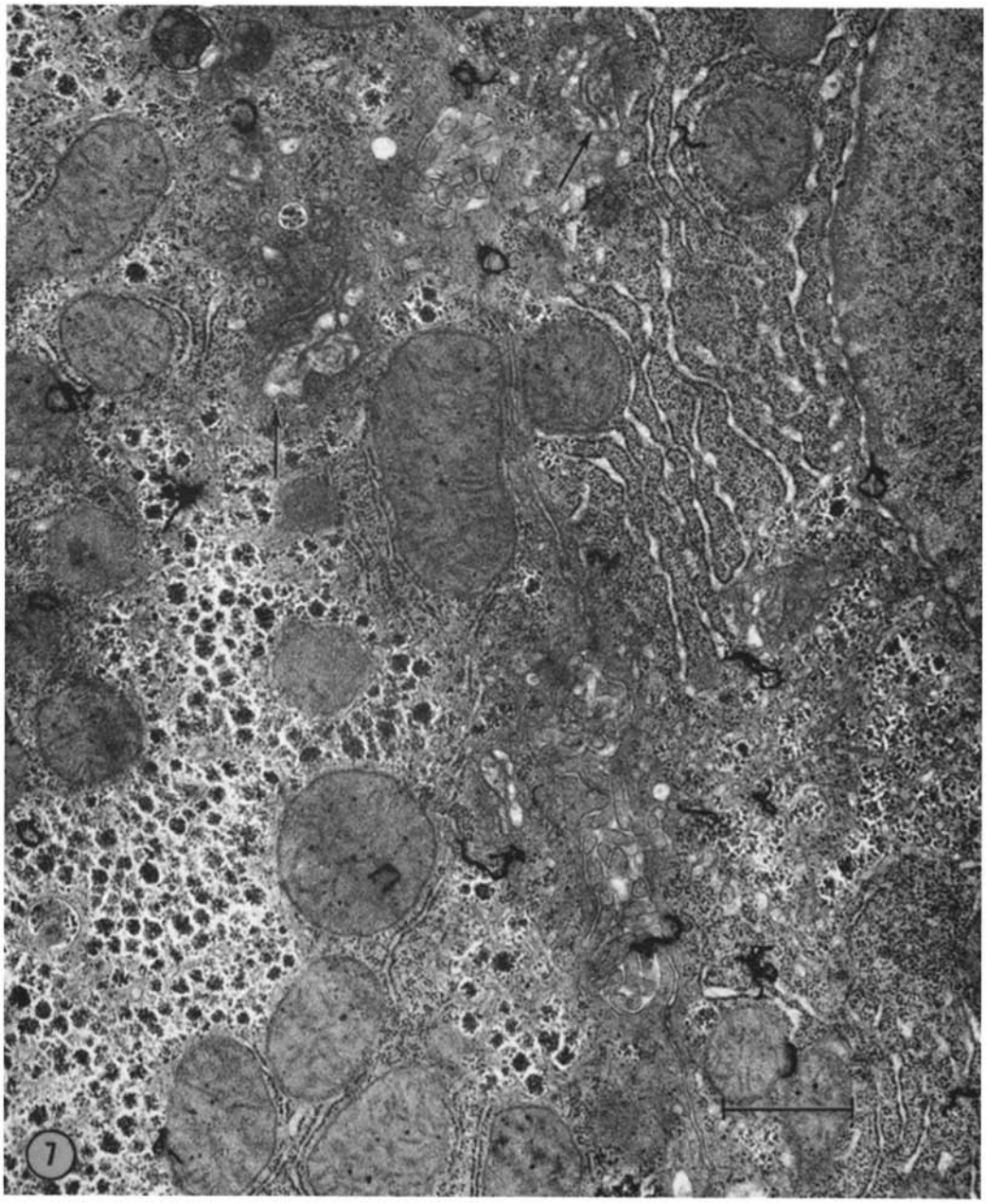
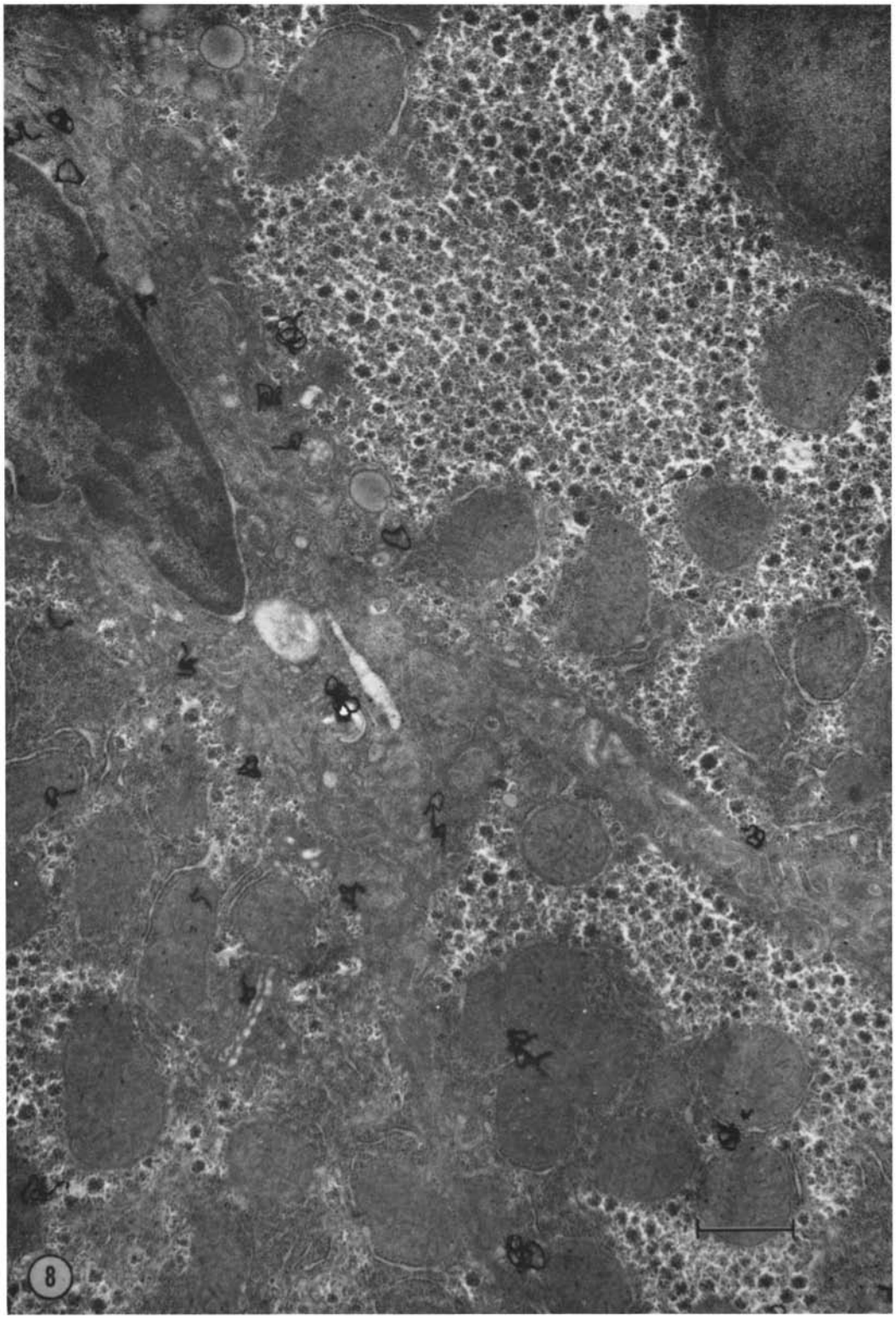


FIGURE 7 5 min after injection. The Golgi apparatus is dilated and in some of the cisternae small granules are seen (arrows). The radioautographic reaction is seen over ER, mitochondria, and "cell boundary." × 18,000.



FIGURES 8 and 9 60 min after injection. Grains are seen over the sinusoidal cell and biliary microvilli, as well as over ER and mitochondria. The lipid droplets and lysosomes are not labeled. Fig. 8, × 15,000; Fig. 9, × 24,000.

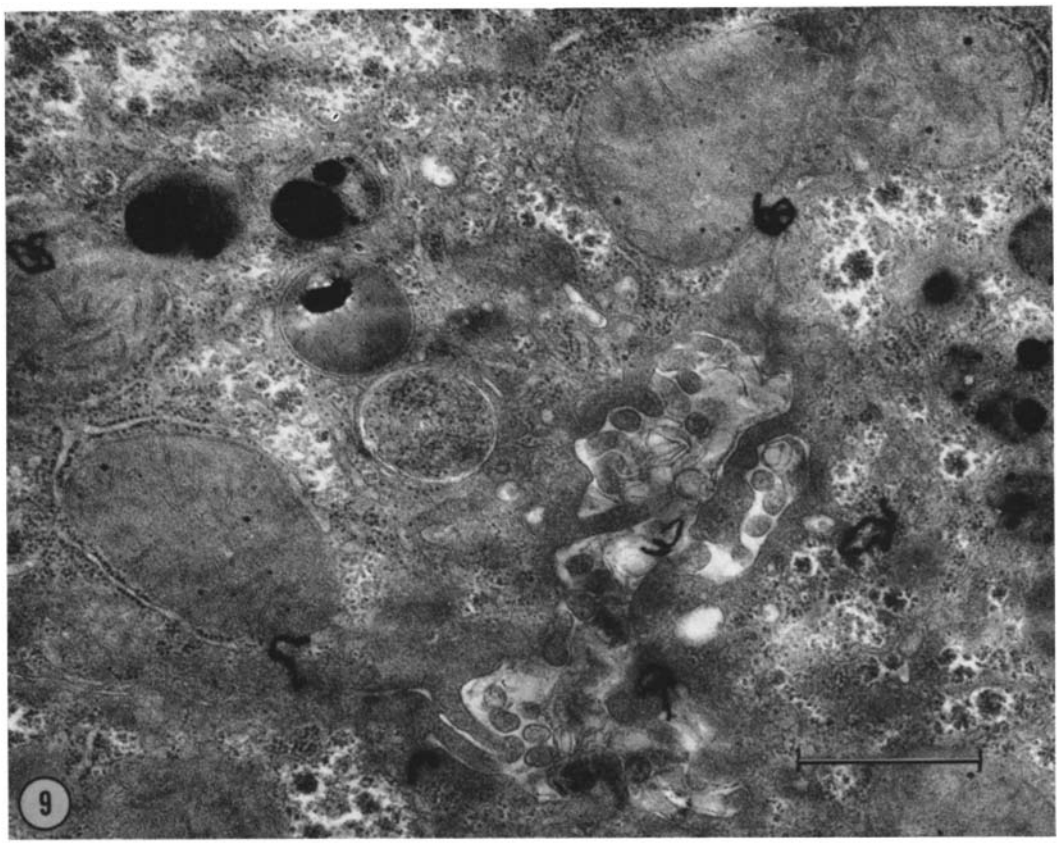
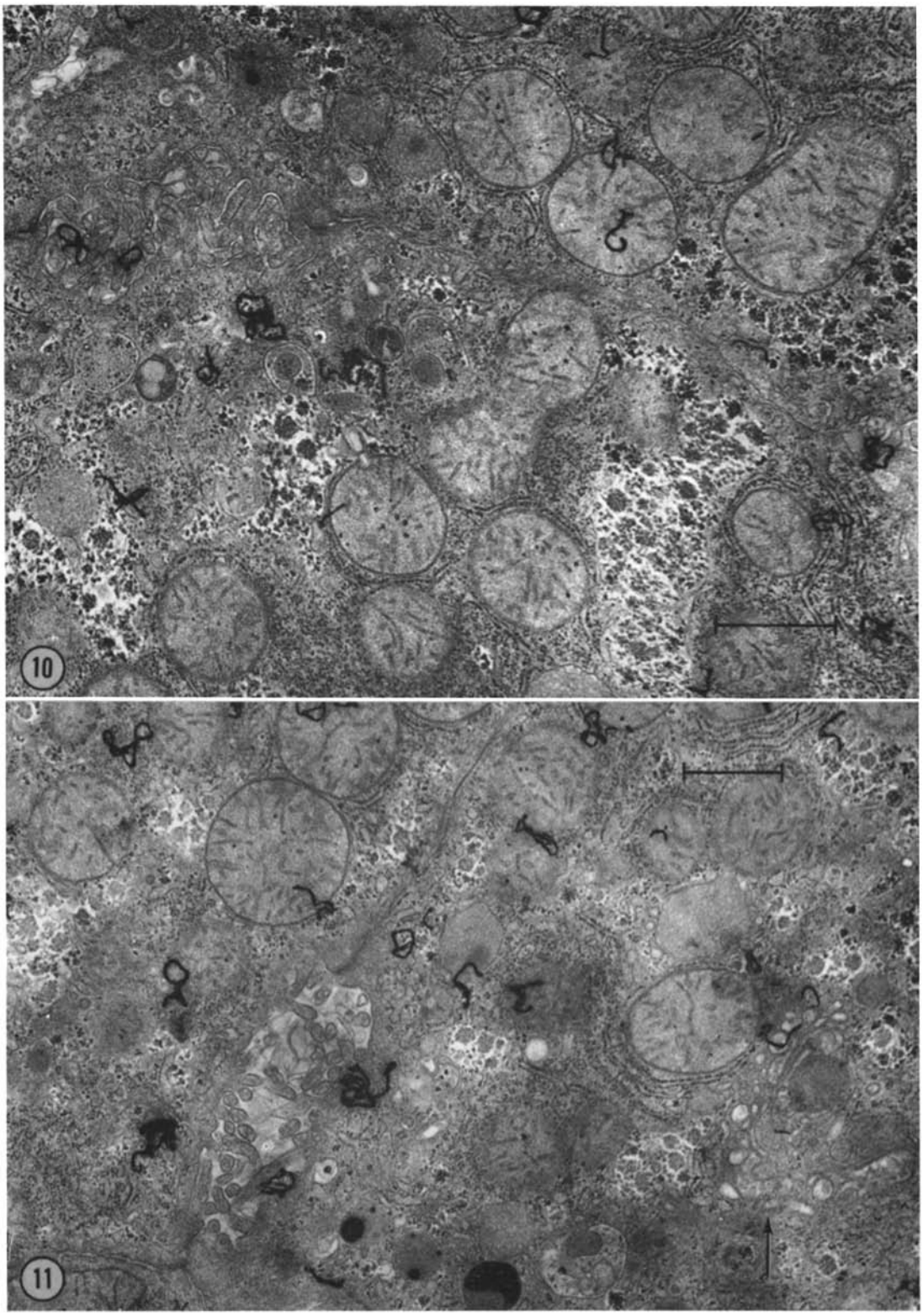


FIGURE 9 See legend under Fig. 8.

bile phospholipid content in the two groups of rats was seen (Table VIII). The 6-hr uptake of lysolecithin-^{3}H by the liver as well as the amount of lecithin-^{3}H which appeared in the serum was the same in both choline-supplemented and choline-deficient rats when expressed as per cent of the 6-hr labeled lecithin-^{3}H content of liver. The choline-deficient rats had a higher incorporation of choline-^{14}C into both liver and biliary lecithin (Table VIII).

The specific activity of biliary lecithin-^{3}H in both choline-deficient and choline-supplemented rats increased slowly after injection of lysolecithin-^{3}H and attained its peak only during the 3rd hour (Fig. 14). It thus seems that the appearance of bile lecithin, derived from lysolecithin, followed mixing with a hepatic pool of lecithin, and that serum lysolecithin cannot be considered as an immediate, direct precursor of bile lecithin. A somewhat different pattern of secretion of bile lecithin-^{14}C after injection of choline was encountered in the two groups of rats. In choline-deficient rats, the peak specific activity of biliary lecithin-^{14}C occurred 1–2 hr after injection, while in the choline-supplemented rats the peak specific activity and the shape of the curves coincided with those of lysolecithin-derived lecithin.

In the choline-deficient rat, more lecithin-^{14}C appeared in the bile, but not in the serum, than in the choline-supplemented rats (Table VIII). This finding suggested that the two lecithins may be derived from different pools. The subcellular site of the precursor pool of bile lecithin was studied in livers perfused in vitro. The experimental design was as follows: livers of 4-day choline-deficient rats were labeled by perfusion for 5–7 min with choline-^{3}H and then were perfused for 120 min with blood containing excess unlabeled choline. The specific activity of the lecithin in bile was compared to the specific activity of microsomal lecithin isolated



FIGURES 10 and 11 The liver was labeled in vivo for 5 min, and liver slices were incubated in vitro with an excess of cold choline for 25 min. There is good preservation of ultrastructure, and the radioautographic reaction is distributed like that found at 5 min. The Golgi apparatus is indicated by arrows. **Fig. 10,** × **18,000; Fig. 11,** × **15,000.**

TABLE VII

Distribution of Radioautographic Reaction over Subcellular Elements in Liver Cells after Labeling with Choline-^3H

In vivo labeling	Incubated in chase	Grains counted	Distribution of grains							
								Cell boundary		
			RER	SER	Mitochondria	Nucleus	Golgi	Bile capillary	Sinusoid	Lateral
min	*min*		%	%	%	%	%	%	%	%
5	0	500	33.8	28.6	13.0	1.4	3.2	7.2	7.8	5.0
5	10	488	37.1	25.3	13.5	1.4	2.8	5.3	11.3	3.3
5	25	430	30.0	30.0	13.0	1.0	3.8	6.6	11.0	4.6
5	115	860	30.2	27.0	17.4	0.6	3.4	6.7	11.7	3.0
60	0	430	31.0	24.1	17.0	1.1	6.0	7.0	11.0	2.8

from a sample of liver at the end of each collection period. As seen in Table IX, the radioactivity appearing in the bile lecithin was highest during the 1st hour and the specific activity reached its maximum at 1 hr. When compared to the specific activity of the total microsomal lecithin, the specific activity of biliary lecithin was higher at all time intervals examined. However the specific activity of Fraction 1, which comprises mostly linoleyl lecithin derived from microsomal lecithin, was similar to that of biliary lecithin. A continuous, linear increase of labeled lecithin in the perfusate was observed (Table IX). Since the perfusate contained serum, the specific activity of the lecithin was not determined. On a cumulative basis, about seven times more labeled lecithin was secreted into the perfusate than into the bile during 2 hr of perfusion.

DISCUSSION

The availability of choline-^3H of high specific activity made it feasible to attempt visualization of an intracellular phospholipid with the help of electron microscopic radioautography. The present results have shown that the elimination of the unreacted precursor from the tissue, before embedding, was satisfactory and that the loss of labeled phospholipid during the processing of the tissue was low. The label in the tissue lipids was found only in lecithin at all time intervals studied. An attempt was made also to test whether in the intact cell choline can be incorporated into lecithin through an exchange reaction which has been described to take place in liver homogenates, to reach its optimum at pH 9, and to be calcium-dependent (7). The results obtained do not support the contention that such an exchange reaction plays any major role either in the liver slice or in the intact rat. This result is in agreement with the results of Bjornstad and Bremer (4), who found that 20 min following the injection of choline-^{14}C the phosphorylcholine had a specific activity 50 times that of the liver lecithin.

Since radioautography at the electron microscope level depends on the amount of label present in the specimen, the rate and extent of incorporation of any precursor become important limiting factors. The pool of free choline in the male rat liver was estimated at 0.14 μmoles per g body weight (31), which would amount to 3.5 μmoles of choline per g of liver in a rat of 100 g. Hence after injection of 0.7 μmoles of choline (equal to 5 mc of the material of highest specific activity), of which 2% were found per g of liver after 5 min, the label became diluted 250 times. An attempt was made, therefore, to reduce this dilution by inducing choline deficiency, in which the estimated free choline pool is reduced to about one-half (31). The by far higher incorporation of label into lecithin, by livers of rats kept on choline-deficient diet for more than 2 days, as compared to normal or rats fed choline-deficient diet for 1 day, could not be explained by the 50% reduction in the total free choline pool. The CDP-choline pool, estimated at 0.05 μmoles/g liver, is not affected by choline deficiency (32), as it is in constant metabolic equilibrium with lecithin (4), and thus would play no role in the increased uptake of choline. It seems more plausible that the free choline of liver does not constitute a homogeneous pool in respect to its availability for lecithin synthesis. A disproportionally greater contraction of the available pool might

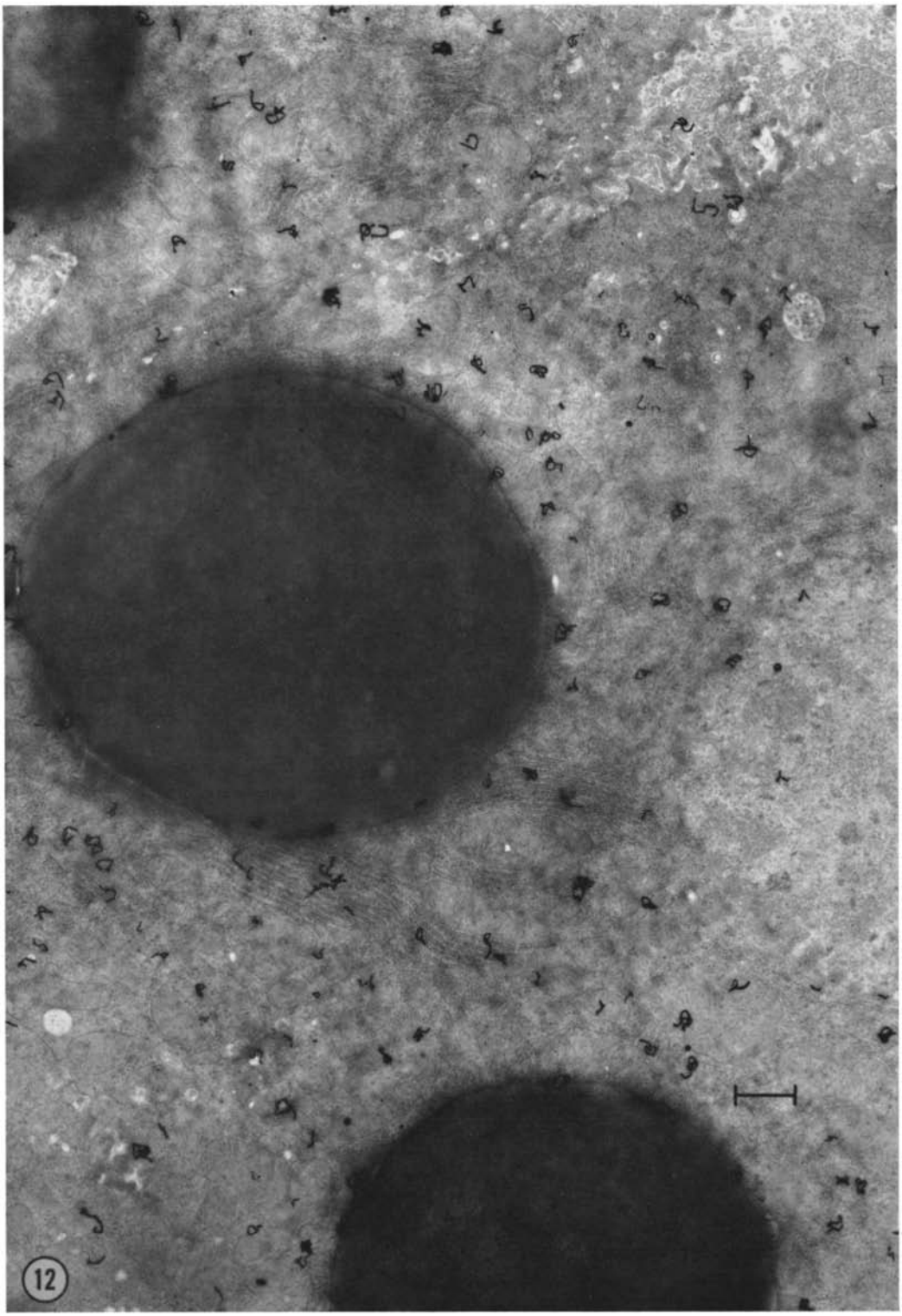


FIGURE 12 Liver slice labeled with choline-^{3}H, incubated in vitro for 120 min. The bulk of the lipid droplets are not labeled, but grains are seen at the droplet boundary. × 9,000.

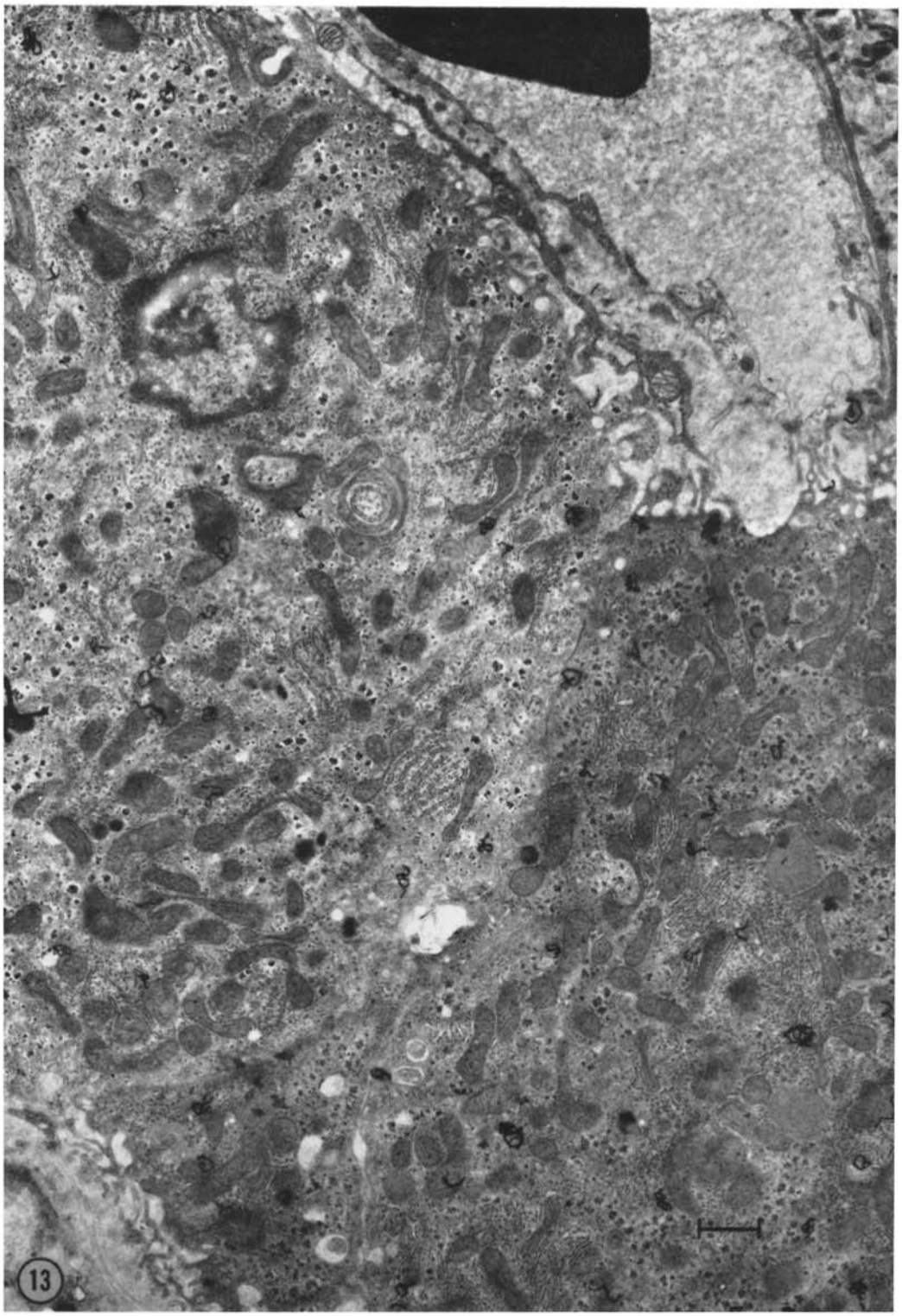


FIGURE 13 Sample of liver of normal rat 120 min after injection of ethanolamine-^{3}H. The radioautographic reaction is seen over ER and mitochondria. There are fewer grains in the region of the sinusoid and biliary microvilli than after injection of choline-^{3}H. × 9,000.

explain the markedly enhanced incorporation of choline into lecithin in choline deficiency. The latter finding is in line with the findings of Zilversmit and Di Luzio (33), who showed a fivefold enhancement of incorporation of ^{32}P into lecithin of choline-deficient dog liver slices, incubated with choline, when compared to slices of control animals.

In the present study, the secretion of lecithin into the bile and the bile flow were similar in choline-deficient and choline-supplemented rats. Using younger rats kept on the choline-deficient diet for a longer period of time and comparing them to rats fed Purina chow, Wells and Buckley (34) found a fall in the secretion of phospholipids into the bile in choline deficiency. This discrepancy might be due to the effect of diet on bile lipids as described by Colwell, who showed that the lipid content of

TABLE VIII

Incorporation of Choline-^{14}C and Lysolecithin-^{3}H into Liver, Biliary, and Serum Lecithin in Choline-Deficient and Choline-Supplemented Rats during 6 Hr after Injection of Labeled Precursors

	Liver*			Bile			Serum‡	
Diet group	Lecithin-^{14}C	Lecithin-^{3}H	Flow	Lipid P	Lecithin-^{14}C‡	Lecithin-^{3}H‡	Lecithin-^{14}C	Lecithin-^{3}H
	%	%	*ml*	μg	%	%	%	%
Choline-supplemented	11.85 ±0.32	36.10 ±2.19	4.40 ±0.95	74.92 ±5.97	0.98 ±0.25	1.42 ±0.38	8.63 ±1.03	9.03 ±0.78
Choline-deficient	21.10 ±1.85	33.40 ±4.90	5.02 ±0.25	72.37 ±12.21	2.17 ±0.77	1.69 ±0.39	9.62 ±0.34	9.40 ±0.35

* Per cent of injected dose in whole liver.

‡ Per cent of labeled lipid in liver 6 hr after injection of precursor. Each group contained four rats. All figures are mean ±SE.

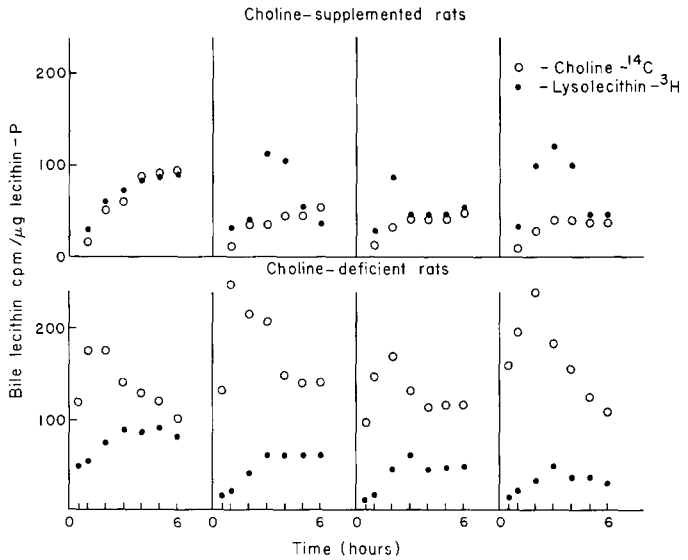


FIGURE 14 Choline-deficient (5 days) and choline-supplemented rats with cannulated bile ducts were injected simultaneously with choline-^{14}C and lysolecithin-^{3}H. The appearance of the label in the bile was measured at ½–1 hr intervals. In the choline-supplemented rats, the shape of the curve of specific activity of bile lecithin was similar after both precursors. In the choline-deficient liver, the peak specific activity of bile lecithin-^{14}C preceded that of the bile lecithin-^{3}H.

TABLE IX

Specific Activity of Lecithin in Bile and Subcellular Fractions of Liver Perfused In Vitro after Labeling with Choline-^3H

Exp.	Time after labeling	Lipid radioactivity			Specific activity of lecithin			
					Microsomes			
		Liver*	Serum‡	Bile§	Total	Fraction I‖	Mitochondria	Bile
	min		*cpm* × 10^{-6}		*cpm* × 10^{-5}/*μg P*			
1	25	73	6.4	1.5	106	*136*	61	*152*
	55	103	18.8	3.4	150	*233*	98	*255*
	85	—	31.6	1.2	—	—	—	246
	115	90	45.1	0.6	125	*160*	123	*214*
2	25	—	7.3	3.2	—	—	—	168
	55	—	24.7	5.0	—	—	—	242
	85	108	32.1	1.2	203	*228*	—	*248*

* Per gram wet weight; ‡ in 100 ml; § in all bile secreted during interval. ‖ Fraction I contains mainly diunsaturated lecithin (see Methods).

bile of rats fed Purina is double that of rats fed the choline-supplemented diet (35).

The secretion of labeled lecithin into the blood stream was studied after injection of choline-^{14}C and lysolecithin-^3H. Although the per cent of labeled liver lecithin appearing in the plasma was similar in both groups of rats, this per cent did not indicate the actual amount secreted. The presence of a smaller lecithin pool in the choline-deficient liver (36, 37) makes such a finding not incompatible with a fall in lecithin secretion into the blood (38, 39).

The much greater avidity of the choline-deficient liver for choline made this liver more suitable for a radioautographic study than the normal liver. Structurally, the livers of 5-day choline-deficient rats differed from the liver of the normal rat mainly by an increase in the amount of glycogen and lipid (30). The increased deposition of glycogen is most probably due to the high sucrose content of the diet. The deposition of lipid occurred in the form of multiple droplets, varying in size from a fraction of a micron to a few microns, which were not bounded by a membrane but often showed a boundary with a density different from the density of the droplet content. After labeling with choline, the bulk of the lipid droplet was not labeled, but radioautographic grains were seen at the boundary of the droplet. Since following injection of a labeled fatty acid, when the label is predominantly in triglycerides, the lipid droplets in liver and mammary gland (28, 40) are uniformly labeled, it seems that, by analogy to the chylomicrons (41), the intracellular lipid droplet has a core containing neutral lipids and an outer coat containing phospholipids. The accumulation of neutral lipids in choline deficiency is due to an impairment of trigyceride secretion by the liver (42, 43, 38, 39) and is accompanied by a fall in the very low density lipoprotein of the plasma (39). In recent studies dealing with the morphology of triglyceride secretion by the liver, the secretory product has been identified as a particle, 50–100 mμ in diameter, which was encountered in the cisternae of the endoplasmic reticulum, in the Golgi apparatus, and in the space of Disse (28, 44, 45). The particles, when isolated from either the perfusate (44, 45) or the Golgi apparatus (46), showed morphological, ultracentrifugal, and chemical characteristics similar to those of the very low density lipoprotein.

In the liver of 5-day choline-deficient rats, it was difficult to find these particles in the space of Disse, and they were few in the Golgi apparatus, thus giving a morphological expression to the impairment of plasma triglyceride secretion.

A study of cellular lecithin must take into account the by now accepted concept of families of lecithins. Collins (47) first showed that two major families of lecithins can be distinguished on the basis of different rates of incorporation of ^{32}P, lecithin rich in linoleic and palmitic acid being more highly labeled than that containing arachidonic

and stearic acids. In choline-deficient rats, the injection of choline promoted the incorporation of ^{32}P into the linoleyl-palmitoyl lecithin (48). More recently, analysis of two groups of lecithins, the dienoic and tetranoic types (based on the presence of linoleic and arachidonic acids, respectively), has shown that, after injection of labeled choline, the former had twice the specific activity of the latter (6, 49). In our experiments, following injection of choline, microsomal lecithin was separated into fractions I and II, corresponding to linoleyl and arachidonyl lecithin, respectively, and about 80% of the radioactivity of the whole lecithin fraction was recovered in the linoleyl fraction. Hence, the radioautographic reaction obtained after the injection of labeled choline was representative predominantly of the linoleyl lecithin. The radioautographic results obtained after injection of labeled choline have shown that from the earliest time intervals studied the main subcellular components labeled were the endoplasmic reticulum, the mitochondria, and the cell boundary, which comprised microvilli and the plasma membrane. The radioautographic reaction at the cell boundary is difficult to interpret but it seems to represent at least two different products, one of which forms part of the cell membrane and the cytoplasmic matrix, the other of which is destined for secretion. This deduction is supported also by the finding of relatively less grains, in these areas, in liver labeled with ethanolamine-^{3}H, the product (PE) of which is not secreted to a large extent, either into the bile or into the serum. In addition, as discussed below, the PE is distributed more slowly between microsomes and mitochondria, and this finding might be also extrapolated to the "cell boundary."

Since about 13% of the label was found over the mitochondria 5 min after injection of choline-^{3}H, and since there was only a small increase with time, both in vivo and in vitro, it seems that mitochondrial lecithin could have been derived from either: (*1*) independent mitochondrial synthesis, or (*2*) lecithin synthesized in the endoplasmic reticulum and transferred rapidly into the mitochondria, or (*3*) a combination of *1* and *2*. Independent synthesis would require the presence of a full complement of enzymes necessary for incorporation of choline into lecithin, in the respective organelle. These enzymes have been localized to the microsomal fraction (1), but recently the incorporation of choline into phospholipids by rat liver mitochondria has been reported (50, 51), and the enzymes active in this reaction were shown to be present in the outer mitochondrial membrane. Stoffel and Schliefer (52) have found CDP-choline transferase activity in the outer mitochondrial membrane, but the activity was about one-tenth of that of the microsomes. Thus, even if mitochondrial synthesis of lecithin would occur in the intact cell, seemingly it would proceed at a rate much slower than that in the microsomes. One would then expect a much higher ratio of specific activity of microsomal to mitochondrial lecithin than that of 1.4–1.8 observed 5 min after injection, irrespective of whether the precursor was choline, glycerol, or linoleate. The same would also be true when lysolecithin is used as precursor, as it is acylated mainly in the microsomes (53, 54). It seems of interest to point out that a similar ratio of specific activity of vesicular to mitochondrial lecithin was observed in *Neurospora*, labeled for 10 min with choline (55). The ratio of specific activity of microsomal to mitochondrial PE, which was 5.5, 5 min after injection of ethanolamine, and fell slowly with time, might be the result of a much slower synthesis of PE in mitochondria than in microsomes. However, as the enzymic pathways of lecithin and PE synthesis are quite similar, a comparably slow mitochondrial synthesis of lecithin would not result in the above-mentioned ratio of 1.4–1.8, and one might invoke on additional process leading to the rapid enrichment of mitochondria with newly synthesized lecithin. The results of the present study seem to indicate that in the intact cell this rapid enrichment could result from the transport of the labeled product from microsomes to mitochondria. In such a process, the ratio of specific activity would be affected by both the rate of transfer and the size of the pool of the labeled product. Hence, if the transport of PE were to occur at a rate similar to that of lecithin transport, one would expect the ratio of specific activity of microsomal to mitochondrial PE to be about 4.0, taking into account the fact that the PE content of mitochondria is higher than that of microsomes (56). Thus, the finding of a higher ratio of specific activity seems to indicate that PE is transported at a slower rate than lecithin. The introduction of new lecithin molecules into preexisting mitochondria in *Neurospora* has been described (55, 57), even though the mechanism by which the phospholipid is added to the structure of the mitochondrion has not been determined. Recently, the occurrence of a rapid exchange of

lecithin between microsomes and mitochondria in vitro has been reported by Wirtz and Zilversmit (58), who indicated also that the exchange of PE occurs at a slower rate. As in these experiments ^{32}P or glycerol-^{14}C served as label of lecithin, a possibility remained that only a part of the molecule, namely lysolecithin, might participate in the exchange. However, our findings of a similar ratio of microsomal and mitochondrial lecithin when glycerol and linoleic acid served as precursors, and of a slight rise in the ratio when lysolecithin was injected, indicate that lysolecithin is not an intermediate in the transfer of microsomal lecithin into mitochondria.

In order to compare data obtained by radioautography to those derived from cell fractionation, the following calculation can be made: On the basis of data of Getz et al. (56), the ratio of microsomal to mitochondrial lecithin content of liver is 3.5. If this ratio is multiplied by the mean ratio of specific activity of microsomal to mitochondrial lecithin (1.53), observed 5 min after injection of labeled choline, glycerol, or linoleate, a ratio of 5.35 is obtained, which represents the expected distribution of radioactivity between microsomes and mitochondria. The actually observed ratio derived from grain counts of radioautographs was 4.80, which fits fairly well with the expected ratio. This calculation is valid even if there is a fall in liver lipid choline content in choline deficiency, since it was shown by Tani et al. (59), for guinea pig liver, that the fall is proportional in microsomes and mitochondria. In the present the ratio of specific activity of microsomal and mitochondrial lecithin did not change during 1 hr, and similar results were obtained also by Bjornstad and Bremer (4), both after injection of labeled choline and after injection of labeled methionine. This lack of complete equilibration in view of the apparent rapidity of the exchange reaction indicates that not all of the mitochondrial lecithin is easily exchangeable It is tempting to speculate that the rapidly exchangeable pool of lecithin is located in the outer mitochondrial membrane and that the more slowy exchangeable pool is residing in the inner mitochondrial membranes.

Similarly, a good fit between the expected ratio of distribution of grains over mitochondria and endoplasmic reticulum of 2.85 and the observed one of 3.0 was found for the 120-min period after injection of ethanolamine-^{3}H.

It is known that after injection of ^{32}P the specific activity of bile lecithin exceeds that of liver lecithin, and it has been postulated that bile lecithin is derived from a separate pool (5). One of the aims of the present study was the elucidation of the site of the lecithin pool from which bile lecithin might originate. It was hoped that if this pool was morphologically separate from the main lecithin pool of the liver, it might become labeled more rapidly and extensively. The radioautographic findings did not reveal any such entity, and most of the label was related to the elements of the endoplasmic reticulum. Since it is not possible by radioautography to distinguish between a lecithin molecule which is a part of a membrane and a lecithin molecule which is destined for secretion, the microsomal fraction had to be isolated and the separation of lecithin effected by chromatography. As shown by others, distinct families of lecithin can be obtained from the microsomal fraction, which vary in their specific activity after injection of labeled choline. As seen in Table VI of reference 6, the specific activity of dienoic lecithin of whole liver was either higher than, or equal to that of dienoic lecithin of bile, up to 2 hr after injection of choline-^{3}H. In the present study, in which the dienoic lecithin was derived from the microsomal fraction, the specific activity of dienoic lecithin was also quite similar to that of bile lecithin, which is mainly linoleyl lecithin (60). Thus, one can conclude that biliary lecithin originates apparently in the endoplasmic reticulum and that the "special pool" can be defined better on a chemical than on a morphological basis. The mechanism by which the linoleyl lecithin is secreted solely into the bile is completely unknown. One could envisage a specific carrier system for binding selectively this type of lecithin and transporting it to the bile. The protein nature of a carrier operative in the exchange of lecithin between liver organelles has been suggested (58).

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Note Added in Proof:

Since the submission of this manuscript, the use of radioactive choline as a selective label for liver microsomal lecithin as well as the nonoccurrence of the choline exchange reaction in rat liver was shown. (Nagley, P., and T. Hallinan. 1968. *Biochim. Biophys. Acta.* **163**:218)

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