# REVERSAL BY ADENINE OF THE ETHIONINE-INDUCED LIPID ACCUMULATION IN THE ENDOPLASMIC RETICULUM OF THE RAT LIVER

## A Preliminary Report

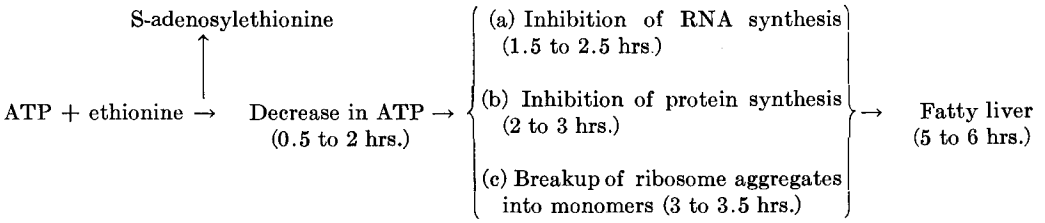
CORRADO M. BAGLIO and EMMANUEL FARBER

From the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

ABSTRACT

Within 3.5 to 4 hours after thionine administration, numerous small osmiophilic bodies, liposomes, appear in the endoplasmic reticulum of the liver cells. By fusion, the liposomes lead to the formation of larger collections of fat, giant liposomes. Adenine administration to ethionine-treated rats removes the liposomes from the hepatocytes and causes the transitory appearance of osmiophilic droplets in the sinusoidal space of Disse. The characteristic disaggregation of hepatic polysomes seen in the liver after ethionine administration is corrected by the injection of adenine.

Ethionine, the ethyl analogue of methionine (9), when administered to female rats, induces a number of biochemical lesions in the liver, the sequence of which is illustrated below (12–14, 26, 27):




The key lesion in this sequence is a rapid and marked decrease in the level of hepatic ATP, which results from the trapping of the adenine moiety of ATP consequent upon the activation of ethionine to S-adenosylethionine. It has been shown, in fact, that the fall in hepatic ATP, the inhibition of protein synthesis, and the development of a fatty liver can be effectively prevented by the simultaneous administration of ATP, or one of its precursors such as adenine, along with the ethionine (12–14, 26, 28). In this report we present, in preliminary form, findings of early ultrastructural changes in the liver cell caused by ethionine, and evidence that these changes, once established, can be reversed by the administration of adenine.

## MATERIALS AND METHODS

White female rats of the Wistar strain (Carworth Farms, New City, New York), weighing from 170 to 200 gm, were fasted overnight. One group of 15 animals was then treated with ethionine (1 mg/gm of body weight); a second group of 6 animals with ethionine and adenine (32 mg per rat); and a third group of 10 animals with saline (8 ml) or adenine. Aqueous solutions of ethionine (25 mg/ml) or adenine (10 mg/ml) were used; these solutions, as well as saline, were injected intraperitoneally. Ethionine or saline was given at zero time, and adenine (or saline) 2.5 to 3.5 hours thereafter. The animals were sacrificed under ether anesthesia at times ranging from 2 to 8 hours after administration of ethionine or saline, and 2 to 3.5 hours after administration of adenine (or saline). Liver tissue was fixed for 2 hours in osmium tetroxide buffered with Veronal-acetate (8), embedded in Epon (18), and cut with a Porter-Blum, MT-1 or Huxley Microtome. Ultrathin sections were stained with uranyl acetate (31) or lead hydroxide (17) and examined with a Philips EM-100 microscope.

## RESULTS

An increasing number of osmiophilic bodies (liposomes Figs. 1 and 2), 500 to 1500 A in diameter, appears within the endoplasmic reticulum of the liver cells 3.5 to 4.5 hours after the administration of ethionine. In the early stages of the experiment these bodies are predominantly located around the sinusoidal border of the hepatocytes, but subsequently are found evenly distributed throughout the cell (Fig. 3). At this stage larger adielectronic bodies (giant liposomes) are also found, and can be seen to derive from coalescence of the smaller ones (Figs. 3 and 4). The number and size of the giant liposomes increase with time, as triglycerides are progressively deposited and accumulate in the liver.

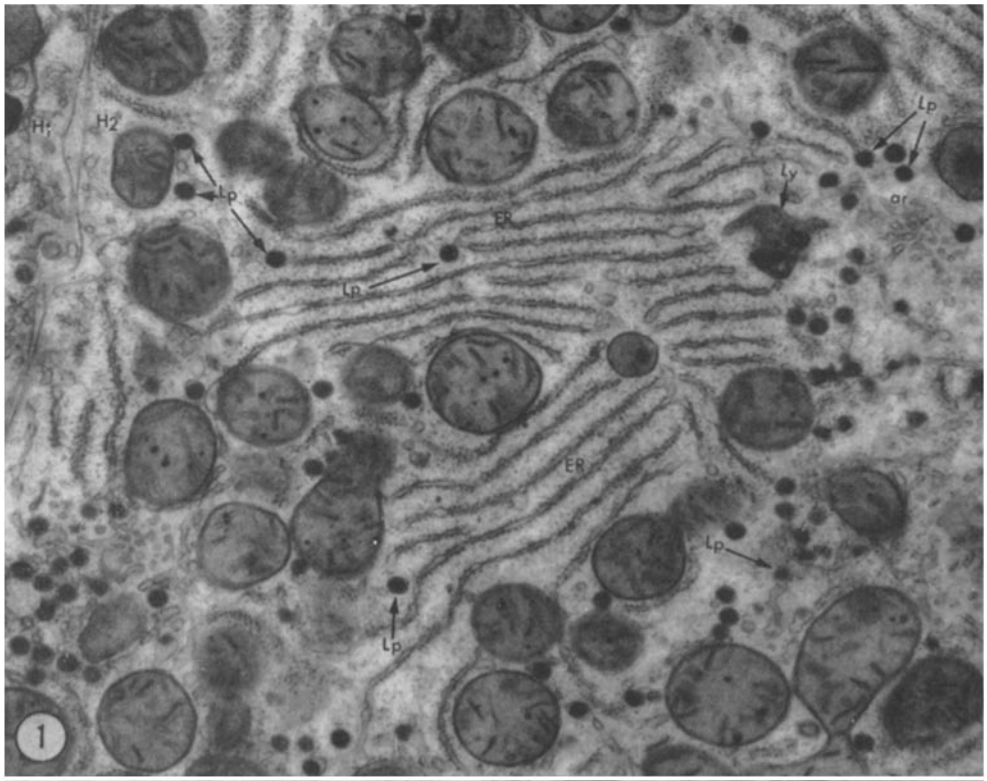
Within 3.5 hours after the administration of adenine to animals injected 3.5 to 4.5 hours previously with ethionine, the liposomes virtually disappear from the endoplasmic reticulum (Figs. 7 and 8), while a large number of osmiophilic droplets can be seen transitorily in the sinusoidal spaces of Disse (Figs. 5 and 6). However, the number and size of the giant liposomes are much less readily influenced by the adenine administration.

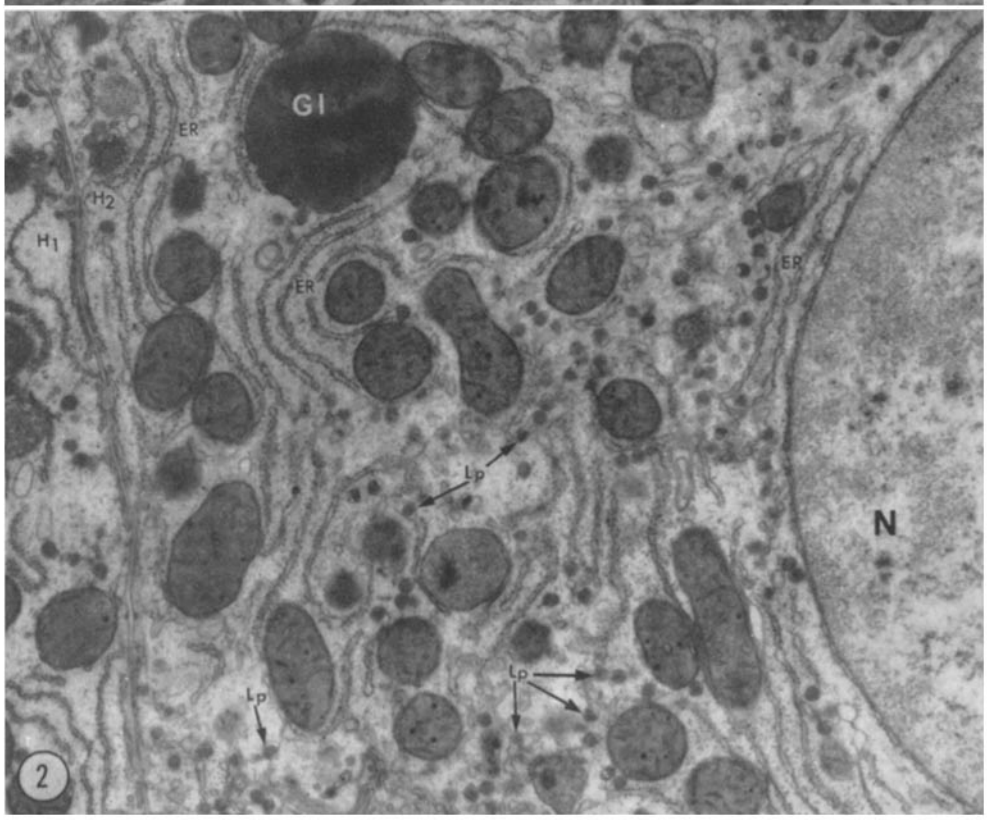
Previous observations have established that in ethionine-treated rats the pattern of ribosomal aggregation, when studied by centrifugation in sucrose gradients, shows a shift from polysomes to ribosomal monomers (27). A similar effect has been observed in the present study. As can be readily seen in Figs. 1, 2, and 4, after the administration of ethionine the ribosomes are dispersed in the hyaloplasm and only single ribosomes or shorter coils remain attached to the endoplasmic reticulum. The dispersion of ribosomes is not associated with an appreciable alteration of the endoplasmic reticulum. The administration of adenine also readily reverses this effect of ethionine (compare Figs. 1, 2, and 4 with Figs. 5, 7, and 8). The Golgi complex of normal hepatocytes contains numerous osmiophilic bodies whose relationship to the liposomes is at present unknown. The modulation of

*Key to Symbols*

*ar*, agranular reticulum
*Ce*, centriole
*E*, endothelial cell
*ER*, endoplasmic reticulum
*Gl*, giant liposome
*G*, Golgi
*H*, hepatocyte
*Lp*, liposome
*Ly*, lysosome
*Mc*, microbody
*N*, nucleus
*Od*, osmiophilic droplet
*Pc*, perisinusoidal canal
*Pr*, perisinusoidal recess
*rc*, ribosomal coil
*rf*, ribosomal figure
*Ss*, sinusoidal space

FIGURES 1 and 2 These two electron micrographs show portions of two hepatocytes (*H1* and *H2*) 4 (Fig. 1) and 4½ hours (Fig. 2) after ethionine administration. The liposomes (*Lp*) are enclosed by a membrane which is frequently continuous with the granular (*ER*) or agranular (*ar*) reticulum. Note the absence of ribosomal coils and the dispersal of ribosomes. A giant liposome (*Gl*) is seen near the upper margin in Fig. 2. Fig. 1, × 18,000; Fig. 2, × 13,000.


H1
H2
Lp
ER
Ly
or
Lp
Lp
ER
Lp
Lp
1


GI
ER
H2
H1
ER
ER
Lp
N
Lp
Lp
2

this complex under similar experimental conditions will be described in a future communication (4).

## DISCUSSION

The presence of osmiophilic bodies in the liver cells has been noted in the early stages of hepatic regeneration (16, 24), in the guinea pig (7) and salamander liver (25), and following the administration of dimethylnitrosamine (11, 21), cerium (19), or ethanol (1). In the present study only a few osmiophilic bodies were seen in the endoplasmic reticulum of control rats treated with saline (Fig. 9), but large numbers were found in the liver of animals treated with ethionine (Figs. 1 to 4). Novikoff *et al.* (22) have presented histochemical evidence that similar osmiophiiic bodies appearing after feeding a diet supplemented with orotic acid are lipid in nature. Additional evidence suggesting their lipid nature is as follows: (*a*) the close association of these bodies with abnormal accumulation of triglyceride in the liver in various pathological conditions; (*b*) a close correlation between the time sequence of the increase in liver triglyceride level (12) and the appearance of these bodies; and (*c*) the fact that the larger adielectronic bodies, which have been recognized for years as lipid in nature, are obviously derived by fusion of the small osmiophilic bodies (Figs. 3 and 4). In view of this evidence the names "liposomes" and "giant liposomes" seem appropriate to indicate the two types of osmiophilic bodies. The name liposomes has been previously applied by other authors (6, 15) to the lipid-formed bodies in the adrenal cortex (32).

After ethionine, the increase in the number of liposomes occurs just prior to a measurable net increase in liver triglyceride (3). Since the basis for the triglyceride accumulation in the liver after ethionine is probably a block in the synthesis of lipoproteins by the liver or in the transfer of this plasma protein to the blood (14), it may be inferred that the engorgement of the endoplasmic reticulum by the osmiophilic bodies is probably the earliest morphological expression of the interference in lipoprotein metabolism.

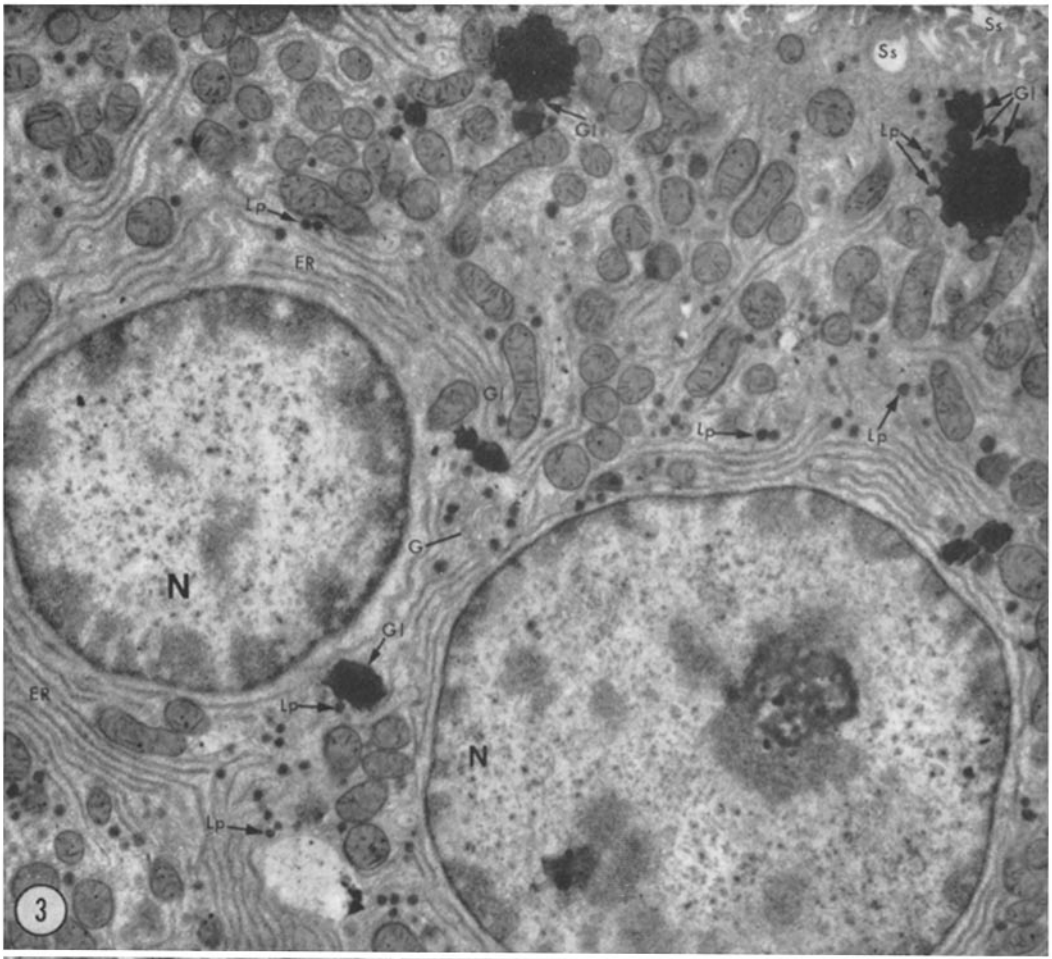
The formation of helical ribosomal aggregates (Fig. 8), following adenine administration to ethionine-treated animals, indicates that the more complex geometrical arrangement of ribosomes described in other tissue (5, 29) and in plant tissue (10) can also occur in the liver, at least under some experimental conditions. It is generally considered that the polysomes are the biological units for the synthesis of proteins (30, 33). The decrease in protein synthesis has been correlated with the disaggregation of polyribosomes during the maturation of erythrocytes (20) and in scorbutic cells during wound healing (23). In our experimental model, the dispersal of ribosomal aggregates occurs after the decrease in ATP and accompanies the inhibition in protein synthesis (27). By counteracting the ATP deficiency induced by ethionine, adenine corrects the disaggregation and dispersal of surface ribosomes (2) with a concomitant reversal in the inhibition in protein synthesis (28). This presumably reconstitutes the lipoprotein packaging mechanism of the cells, with consequent disappearance of liposomes from the endoplasmic reticulum.

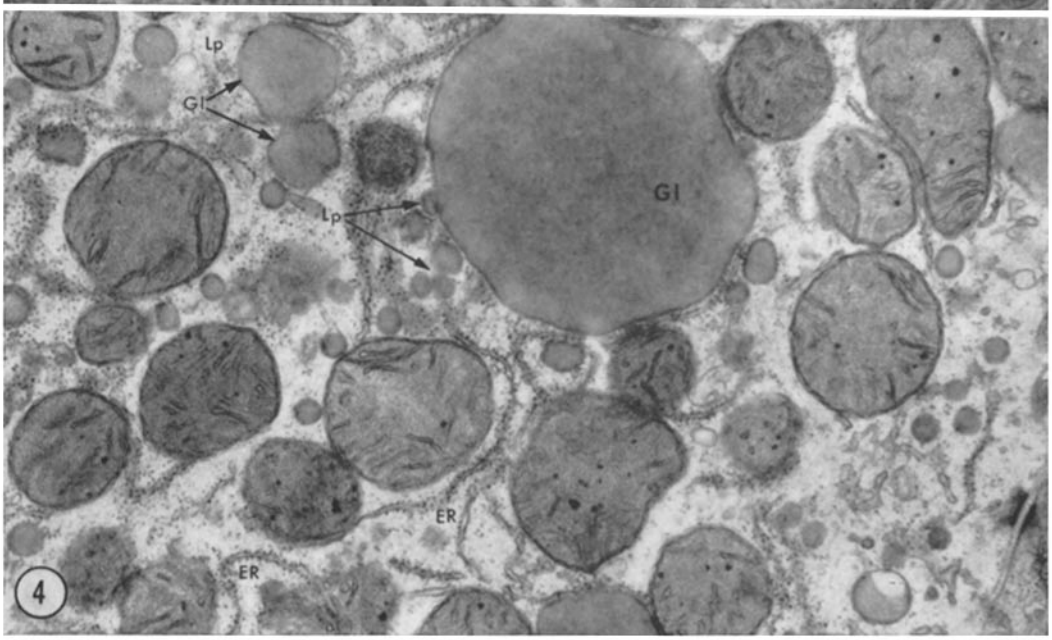
Grateful acknowledgement is made to Miss Rosina Coltellaro and Miss Gloria Diluiso for their able technical assistance, to Mrs. LaRue Wosko for advice and assistance in the photography part of this work, and to Miss Georgetta Marhefka for editing the manuscript.

This work was supported in part by research grants from the United States Public Health Service, Grant No. AM-05590 from the National Institute of Arthritis and Metabolic Diseases, CA-06074 from the National Cancer Institute, GM-10269 from the

FIGURE 3 A binucleated hepatocyte 5 hours after ethionine. Note the lack of polarity of the liposomes (*Lp*) around the sinusoidal space (*Ss*) and the fusion of liposomes (*Lp*) and giant liposomes (*Gl*). × 9000.

FIGURE 4 Portion of a hepatocyte 6 hours after ethionine. Shorter fixation has rendered the liposomes less osmiophilic. Fusion of liposomes (*Lp*) and giant liposomes (*Gl*) is indicated. The ribosomes are dispersed in the hyaloplasm. Only single ribosomes remain attached to the endoplasmic reticulum (*ER*). 22,000.


Ss
Ss
Gl
Gl
Lp
Lp
ER
Gl
Lp
Lp
Gl
N
Gl
ER
Lp
N
Lp
3


Lp
Gl
Gl
Lp
ER
ER
4

National Institute for General Medical Sciences, and a training grant, 2G-135 from the latter institute; the Life Insurance Medical Research Fund, the American Cancer Society and the Beaver County Cancer Society.

*Received for publication, June 15, 1965.*

## REFERENCES

1. ASHWORTH, C. T., WRIGHTSMAN, F., COOPER, B., and DI LUZIO, N. R., Cellular aspects of ethanol-induced fatty liver: a correlated ultrastructural and chemical study, *J. Lipid Research*, 1965, **6**, 258.
2. BAGLIO, C. M., and FARBER, E., Correspondence between ribosome aggregation patterns in rat liver homogenates and in electron micrographs following ethionine administration, *J. Mol. Biol.*, 1965, **12**, 466.
3. BAGLIO, C. M., and FARBER, E., Ultrastructural consequences of biochemical lesions in the liver induced by ethionine, *Fed. Proc.*, 1965, **24**, 556, (abstract).
4. BAGLIO, C. M., and FARBER, E., Ultrastructural consequences of biochemical lesions induced by ethionine in rat liver cells, in preparation.
5. BEHNKE, O., Helical arrangement of ribosomes in the cytoplasm of differentiating cells of the small intestine of the rat foetuses, *Exp. Cell Research*, 1963, **30**, 597.
6. BELT, W. D., The origin of adrenal cortical mitochondria and liposomes. A preliminary report, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 337.
7. CAESAR, R., Electronenmikroskopischer Nachweis von Fettpartikeln im Disseschen Raum, *Z.Zellforsch u. Mikr.Anat.*,1961,54,793.
8. CAULFIELD, J. B., Effects of varying the vehicle of $OsO_4$ in tissue fixation, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 827.
9. DYER, H. M., Evidence of physiological specificity of methionine in regard to methylthiol group: synthesis of S-ethylhomocysteine (Ethionine) and study of its availability for growth, *J. Biol. Chem.*, 1938, **124**, 519.
10. ECHLIN, P., An apparent helical arrangement of ribosomes in developing pollen mother cells of *Ipomoea purpurea* (L.) Roth, *J. Cell Biol.*, 1965, **24**, 150.
11. EMMELOT, P., and BENEDETTI, E. L., Changes in the fine structure of rat liver cells brought about by dimethylnitrosamine, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 393.
12. FARBER, E., LOMBARDI, B., and CASTILLO, A. E., The prevention by adenine triphosphate of the fatty liver induced by ethionine, *Lab. Inv.*, 1963, **12**, 873.
13. FARBER, E., SHULL, K. H., and VILLA-TREVINO, S., The regulation of hepatic activity by adenosine triphosphate (ATP), *in* Symposium on Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic liver, (G. Weber, editor), New York, Pergamon Press Inc., 1963, 259.
14. FARBER, E., SHULL, K. H., VILLA-TREVINO, S., LOMBARDI, B., and THOMAS, M., Biochemical pathology of acute hepatic adenosine triphosphate deficiency, *Nature*, 1964, **203**, 34.
15. IDELMAN, S., Mitochondries et liposomes description d'une transformation mitochondriale observée dans la cortico-surrénale du rat, *J. Micr.*, 1964, **3**, 444.
16. JORDAN, S. W., Electron microscopy of hepatic regeneration, *Exp. Mol. Path.*, 1964, **3**, 183.
17. KARNOVSKY, M. J., Simple methods for "staining with lead" at high pH in electron microscopy, *J.Cell Biol.*, 1961, **11**, 729.
18. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
19. MAGNUSSON, G., The behavior of certain lanthanons in rats, *Acta Pharmacol. et Toxicol., Suppl.*, 1963, **20**, 1.
20. MARKS, P. A., RIFKIND, R. A., and DANON, D., Polyribosomes and protein synthesis during reticulocyte maturation *in vitro*, *Proc. Nat. Acad. Sc.*, 1963, **50**, 336.
21. MUKHERJEE, T., GUSTAFSSON, R. G., AFZELIUS, B. A., and ARCHENIUS, E., Effects of carcinogenic amines on amino acid incorporation by liver systems. II. Morphological and biochemical study on the effects of dimethylnitrosamine, *Cancer Research*, 1963, **23**, 944.
22. NOVIKOFF, A. B., ROHEIM, P. S., and QUINTANA, N., Lipid in the liver cells of rats fed orotic acid and adenine, *Fed. Proc.*, 1954, **23** 126, (abstract).
23. ROSS, R., and BENDITT, E. P., Wound healing and collagen formation. IV. Distortion of ribosomal pattern in scurvy, *J. Cell Biol.*, 1964, **22**, 365.
24. TROTTER, N. L., A fine structure study of lipid in mouse liver regenerating after partial hepatectomy, *J. Cell Biol.*, 1964, **21**, 233.
25. VAN BREEMEN, V. L., Ultrastructural relationships of membranes and vesicles, *Anat. Rec.*, 1964, **148**, 421, (abstract).
26. VILLA-TREVINO, S., and FARBER, E., The reversal by adenosine triphosphate of ethionine-induced inhibition of protein synthesis, *Biochem. et Biophysica Acta*, 1962, **61**, 649.

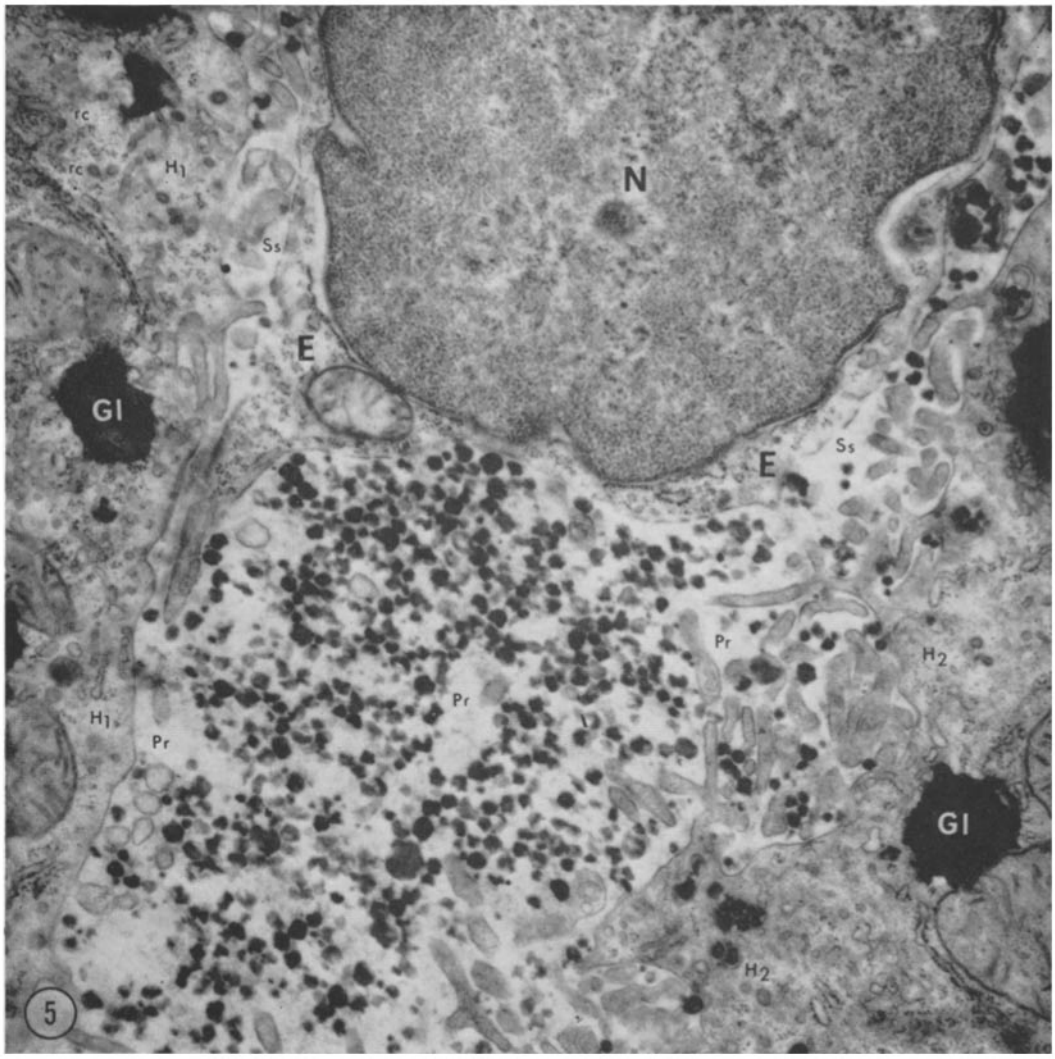
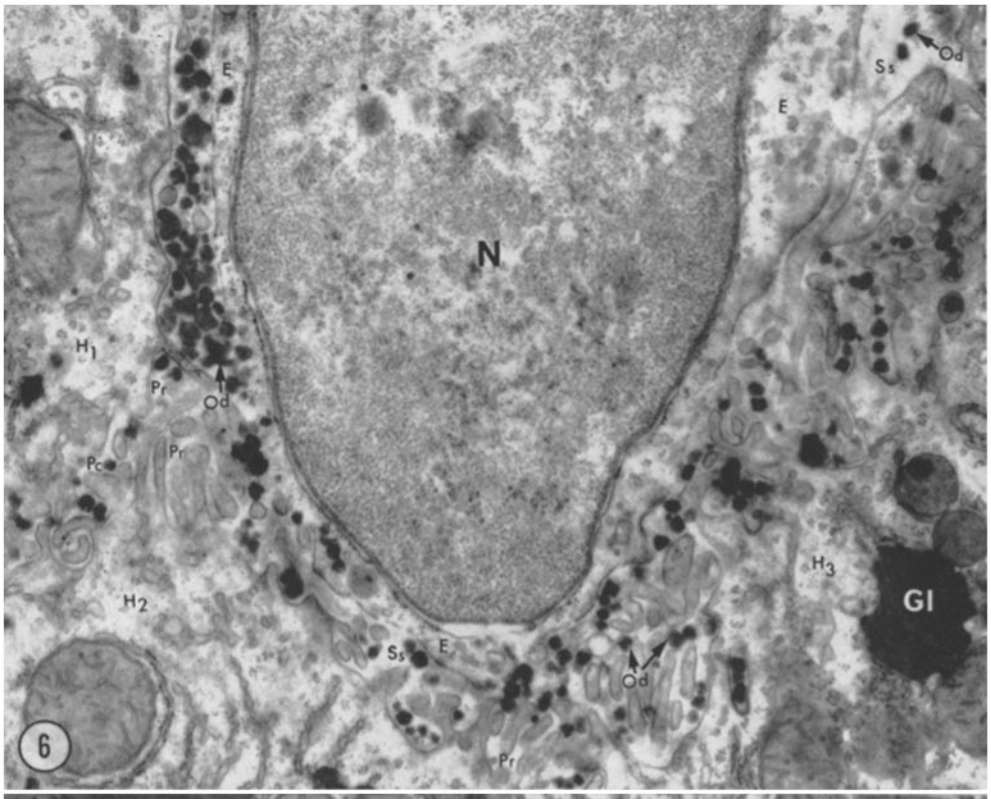


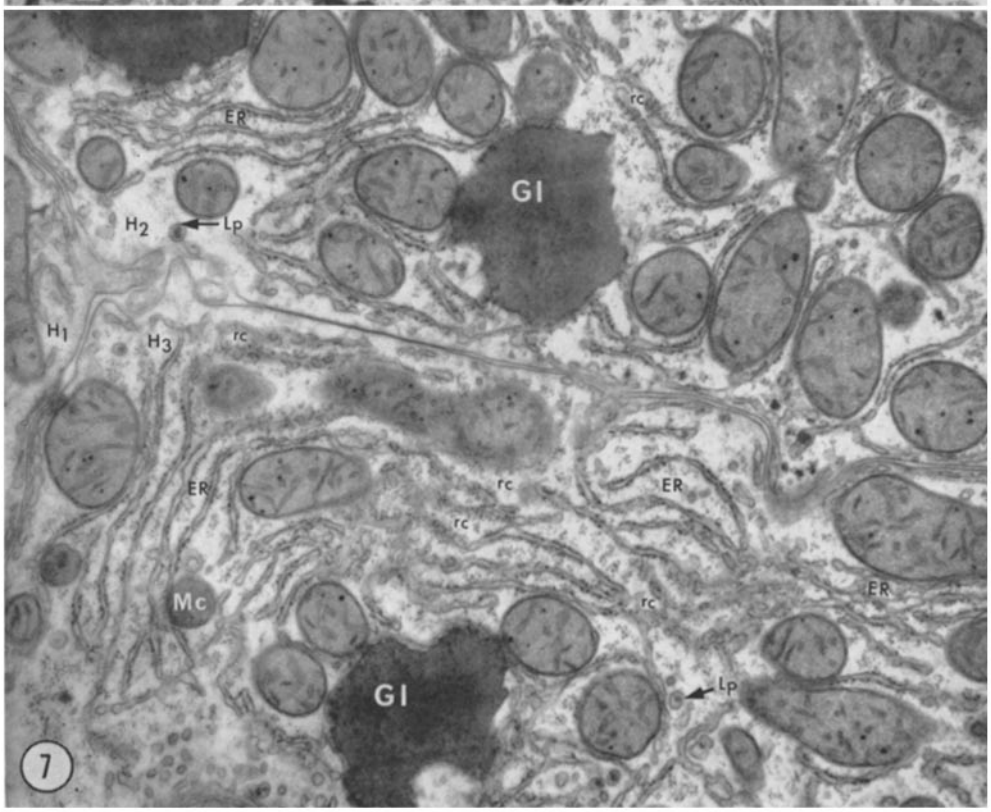

FIGURE 5 Portion of two hepatocytes (*H1* and *H2*) and an endothelial cell (*E*), 4 hours after ethionine and 3½ hours after adenine, delimiting a perisinusoidal recess (*Pr*). Note the large number of osmiophilic droplets in the perisinusoidal recess (*Pr*) and sinusoidal space (*Ss*). Ribosomal coils (*rc*) are indicated in the upper left corner. × 18,000.

27. VILLA-TREVINO, S., FARBER, E., STAEHELIN, T., WETTSTEIN, F. O., and NOLL, H., Breakdown and reassembly of rat liver ergosomes after administration of ethionine or puromycin, *J. Biol. Chem.*, 1964, **239**, 3826.
28. VILLA-TREVINO, S., SHULL, K. H., and FARBER, E., The role of adenosine triphosphate deficiency in ethionine-induced inhibition of protein synthesis, *J. Biol. Chem.*, 1963, **238**, 1757.
29. WADDINGTON, C. H., and PERRY, M. M., Helical arrangement of ribosomes in differentiating muscle cells, *Exp. Cell Research*, 1963, **30**, 599.
30. WATSON, J. D., Involvement of RNA in the synthesis of proteins. The ordered interaction of three classes of RNA controls the assembly of amino acids into proteins, *Science*, 1963, **140**, 17.
31. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.
32. WEBER, A. F., USENIK E. A., and WHIPP, S. C., Experimental production of electron dense intramatrical bodies in adrenal zona glomerulosa cells of calves, *in* Electron Microscopy, 5th International Congress for Electron Microscopy, Philadelphia, 1962, (S. S. Breese, Jr., editor), New York, Academic Press Inc., 1962, **2**, YY-7.
33. WETTSTEIN, F. O., STAEHELIN, T., and NOLL, H., Ribosomal aggregate engaged in protein synthesis: characterization of ergosome, *Nature*, 1963, **197**, 430.

FIGURE 6 Electron micrograph of liver 3½ hours after ethionine and 1½ hours after adenine. A sinusoidal space (*Ss*), delimited by three hepatocytes (*H1*, *H2*, and *H3*) and one endothelial cell (*E*), contains numerous osmiophilic droplets (*Od*). Note also osmiophilic droplets between the villi, in the perisinusoidal recess (*Pr*), and in the perisinusoidal canal (*Pc*). × 16,000.

FIGURE 7 Portions of three hepatocytes (*H1*, *H2* and *H3*), 4 hours after ethionine and 3½ hours after adenine, exhibiting only an occasional liposome (*Lp*). Note now the reconstitution of ribosomal coils (*rc*) and their association with the endoplasmic reticulum (*ER*). × 19,000.


N
E
E
Ss
Od
H1
Pr
Od
Pc
Pr
H2
H3
Gl
Ss
E
Od
Pr
6


ER
rc
Gl
H2
Lp
H1
H3
rc
ER
rc
ER
rc
ER
Mc
rc
Gl
Lp
7

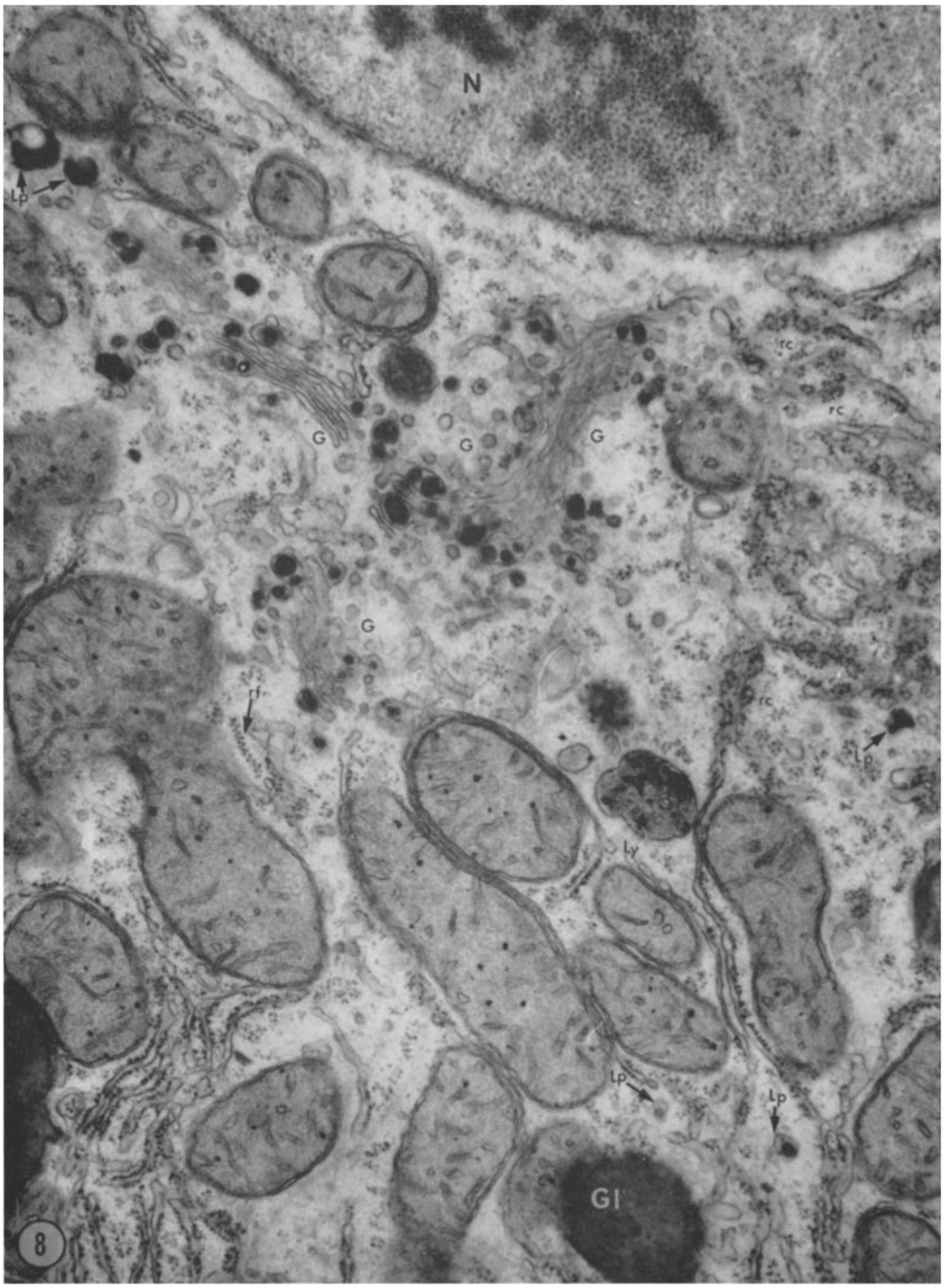



FIGURE 8 Portion of hepatocyte 4 hours after ethionine and 3½ hours after adenine. Five liposomes (*Lp*) are indicated, but note that a complement of osmiophilic bodies is associated with each Golgi complex (*G*). The ribosomes are arranged in long coils (*rc*) and helical figures (*rf*). × 39,000.

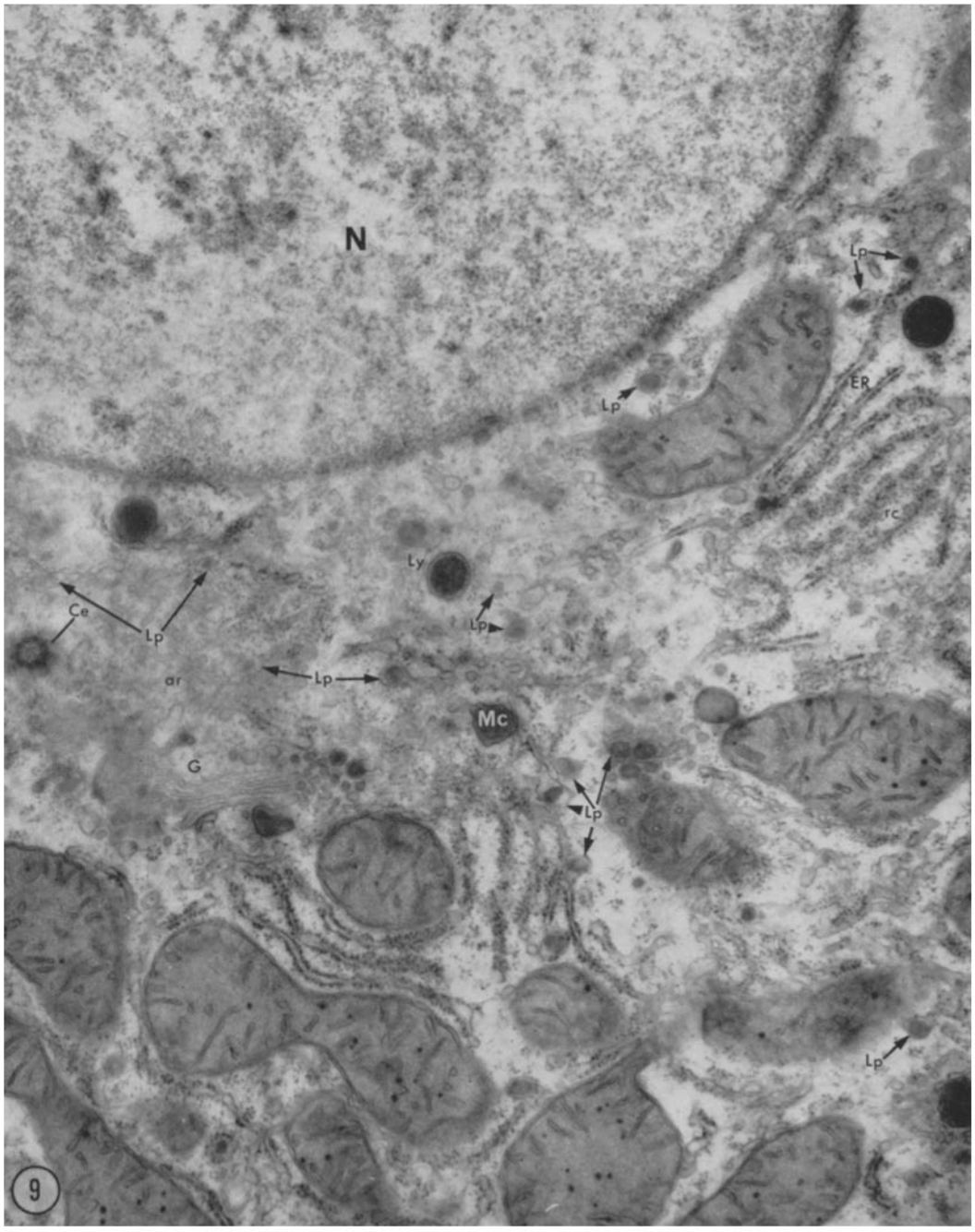



FIGURE 9 Portion of an hepatocyte of a control rat showing liposomes (*Lp*), most of which are within the granular (*ER*) or agranular (*ar*) reticulum. Note the geometrical arrangement of the ribosomes (*rc*) and their association with the cisternae of the endoplasmic reticulum. Osmiophilic bodies are associated with the tangentially cut Golgi complex (*G*). A microbody (*Mc*) is continuous with a tubular profile. × 25,000.