Ultrastructural and physiological evidence for corticosteroid-induced alterations in hepatic production of very low density lipoprotein particles

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Abstract The cause of corticosteroid-induced hyperlipoproteinemia was studied in rats and mice. An ultrastructural morphometric method was utilized to demonstrate alterations in hepatocyte very low density lipoprotein content, and Triton WR 1339-treated rats were used to identify changes in the removal of very low density lipoproteins from plasma. The results show that corticosteroid treatment results in (*1*) an increase in both plasma triglyceride and cholesterol levels, (*2*) an increase in rate of accumulation of triglyceride after inhibition of very low density lipoprotein removal by Triton, and (*3*) an increase in the number and size of Golgi-associated very low density lipoprotein particles in hepatocytes. These combined results suggest that corticosteroids induce hyperlipoproteinemia through increased hepatic production of very low density lipoproteins.

Supplementary key words hypertriglyceridemia · hypercholesterolemia · Triton WR 1339 · Golgi apparatus

During the past 20 yr the effect of adrenal cortical hormones on various aspects of lipid metabolism has been studied in various animal species under a wide variety of experimental conditions (1–13). This has resulted in a number of conflicting conclusions on even such a simple question as to whether corticosteroids increase plasma triglyceride and cholesterol levels. In fact, in a recent review on the subject Rudman and Di Girolamo (14) have evaluated this question and concluded that corticosteroids *regularly* raise plasma lipid levels only in rabbits and chickens.

Furthermore, even in those studies in which corticosteroids have been shown to increase plasma lipids, little information is available as to the mechanisms responsible for the hyperlipemia. Hill, Droke, and Hays (9) have shown that the accumulation of plasma triglycerides 24 or 48 hr after the administration of Triton WR 1339 was greater in two cortisone-treated rats than in two control rats studied. In an electron microscope study, Mahley and coworkers (11) have described an apparent increase in the number and size of very low density lipoprotein particles in the hepatocyte Golgi apparatus and plasma of rabbits given cortisone for 4–6 days. Although these results are consistent with the hypothesis that corticoids produce hyperlipemia by increasing production of very low density lipoproteins, they do not appear to offer conclusive evidence for this possibility. Therefore, in an effort to provide additional information as to the cause of corticoid-induced hyperlipemia we have carried out the current investigation. In this instance we have studied two species of animals (rats and mice) and have refined and combined the ultrastructural and physiological approaches used in the earlier studies. Our results show that corticosteroid administration can increase plasma triglyceride and cholesterol levels in both rats and mice. These corticoid-induced changes in lipid levels were associated with an increase in size and number of VLDL particles present in hepatocyte Golgi from corticoid-treated animals and an increase in accumulation of triglyceride in the plasma after administration of Triton WR 1339. These various observations, when taken together, strongly suggest that increased hepatic lipoprotein production is the mechanism responsible for corticosteroid-induced lipemia.

METHODS

Experimental protocol

Young adult female Sprague-Dawley rats (175–199 g) and female mice of the C3H strain (19–21 g) were housed

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Abbreviations: VLDL, very low density lipoproteins; MP, methylprednisolone; TG, triglyceride.

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four to a cage and allowed to eat (Wayne Lab Blox) and drink ad lib. for 2 wk prior to the onset of experimental procedures. Room temperature was controlled and room lights were automatically turned on at 6 a.m. and off at 6 p.m. Animals received intramuscular injections (0.6 mg/100 g) of either methylprednisolone (Depo-Medrol [20 mg/cc], Upjohn Co., Kalamazoo, Mich.) or an equal volume of saline on days 1, 3, 5, and 8. The feeding schedule was based upon the results of preliminary studies in which the daily food intake of control and corticoid-treated rats was determined. Based upon these observations each rat received a daily ration of chow, 15 g/rat, which would be fully consumed by all rats. The chow was placed in each rat cage every morning until 8 a.m. on day 9, at which time food was removed from the cages, and the animals were killed 5 hr later. This interval of time was adopted because lipoprotein electrophoresis (15) performed during planning studies indicated that chylomicrons were absent from plasma of control rats and mice and only began to appear in trace amounts in corticoid-treated animals when triglyceride levels were very high (>300 mg/100 ml). Control rats gained an average of 10% of initial body weight while consuming 15 g of rat chow/day, while corticoid-treated rats lost an average of 19% of initial body weight on the same diet.

In some instances rats were injected with Triton WR 1339 (16–18) as follows. 5 hr after food had been removed on day 9 the animals were lightly anesthetized with ether, and 1 ml of blood was removed by cardiac puncture for measurement of base-line triglyceride and cholesterol concentrations. The rats then received 600 mg of Triton/kg body weight intravenously. 2 hr later the animals were killed, and blood was obtained again for measurement of plasma triglyceride and cholesterol levels. The dose of 600 mg of Triton WR 1339/kg body weight was based upon preliminary experiments that indicated that the triglyceride levels of this group of normal animals increased linearly with increasing doses of Triton up to 600 mg/kg body weight, after which further increases in Triton concentration did not lead to any further increases in triglyceride levels.

Electron microscopic examination of liver tissue from rats given 600 mg of Triton/kg body weight 2 hr before death showed no morphological changes connected with the administration of this detergent. In addition, morphometric analyses (see below) of the two largest hepatocyte Golgi complexes from three cells of four Triton-treated rats revealed normal values for the mean (±SE) number (112 ± 12 VLDL/Golgi) and diameter (51.5 nm) of VLDL particles present.

Finally, the plasma volumes of six control and six adrenal corticoid-treated rats were estimated by standard dye dilution techniques after the intravenous injection of Evans blue.

Chemical procedures

Blood was collected in tubes containing EDTA and immediately centrifuged, and the plasma was separated and stored frozen. Plasma triglyceride and cholesterol concentrations were determined on an AutoAnalyzer (19, 20). In separate experiments we were able to show that the addition of Triton WR 1339 directly to plasma did not affect these measurements.

Preparation of liver tissue for electron microscopy

Tissue samples from each mouse and rat were taken from a slice through the approximate center of the left lobe of the liver. The tissue was routinely fixed in 1% osmium tetroxide in Millonig's phosphate buffer (pH 7.2) for 3 hr at 4°C, dehydrated in graded alcohols, and embedded in Epon–Araldite plastic. The blocks were trimmed to include cells in the portal–midzonal areas; silver-gray thin sections were prepared, stained with Reynolds lead citrate for 60 min, and examined with a Hitachi HS-8 electron microscope. An alternative fixation schedule was used for a few liver samples from both control and corticosteroid-treated rats. These tissues were fixed in osmium as above, then postfixed in 0.1 M uranyl acetate (pH 5.8) for 30–60 min. Although this method minimized glycogen staining as intended, it appeared to extract and deform a substantial number of VLDL particles and was therefore not used routinely in this study.

In order to compare the effect of adrenal corticosteroid treatment on the number and size of VLDL in the liver, the following morphometric procedure was adopted to take advantage of the fact that large numbers of VLDL particles are clustered together in vesicles associated with Golgi complexes. Three randomly selected, nucleated hepatocytes from each of five corticosteroid-treated and five control rats were examined with the electron microscope at an original magnification of 3000 ×. At the time of examination the identity of the animal from which the liver sections were taken was not known to the microscopist. Each selected cell was mapped out on a sheet of paper and the cellular position of every Golgi complex was identified. At higher magnification all VLDL particles present in these identified Golgi complexes were counted. In this way the number of Golgi present and the total Golgi VLDL particles present per hepatocyte were obtained. In addition, two Golgi complexes estimated to be the largest in each of seven cells were photographed at 12,000 × their original size. These negatives were photographically enlarged to a final magnification of 28,000 × and examined with a 10 × ocular. The diameters of all particles with sufficiently clear outlines were measured in millimeters with an eyepiece reticule. This meant that a variable number of existing particles were measured within each Golgi. Between 287 and 721 particles were measured per rat in order to obtain the mean VLDL-particle size.

TABLE 1. Effect of Triton WR 1339 on plasma triglycerides in control and methylprednisolone-treated rats

Group	Number of Rats	Before Triton	2 hr after Triton	Increment in 2 hr	Plasma Volume	Estimated Rate of TG Entry into Plasma Compartment
		mg TG/ml plasma			*ml*	*mg/min*
Control	6	0.74 ± 0.09	12.77 ± 0.66	12.03	9.7 ± 0.9	0.97
MP-treated	6	2.44 ± 0.54	22.27 ± 1.35	19.83	8.9 ± 0.5	1.45[a]

Values are means ± SE.
[a] MP-treated greater than control, $P < 0.01$.

In mice, estimates of both VLDL number and size were obtained from the two largest Golgi obtained from seven cells of each of five corticosteroid-treated and control animals.

RESULTS

Effect of adrenal corticosteroid treatment on plasma and hepatic lipoprotein metabolism in rats

Administration of methylprednisolone for 8 days to normal rats resulted in increased plasma levels of triglyceride and cholesterol (Fig. 1). Although the percentage increase in plasma triglyceride level (148%) was approximately twice the increase in plasma cholesterol (72%), the changes in both lipids were statistically significant. Since chylomicron accumulation could not account for the hypertriglyceridemia (see Experimental Protocol), the observed changes in plasma triglyceride and cholesterol concentrations suggest that the rise in plasma lipids was due to an increase in plasma VLDL concentration.

In order to gain insight into the cause of the hyperlipoproteinemia, we took advantage of the ability of Triton WR 1339 to inhibit the removal of lipoproteins from plasma (17, 18). With this approach, and by measuring the accumulation of lipids in the plasma after injection of Triton WR 1339, it was possible to get some idea as to whether the rise in plasma lipid levels was due primarily to increased lipoprotein production or decreased lipoprotein removal. We did this by measuring plasma triglyceride and cholesterol levels before and 2 hr after administration of Triton WR 1339. These results are summarized in Table 1 and indicate that the post-Triton accumulation of triglycerides in the plasma of corticoid-treated rats is substantially greater than in control rats. It should be noted in Table 1 that plasma triglyceride levels rose in the corticoid-treated rats proportionately more than did estimates of triglyceride entry rates. This relationship suggests that normal mechanisms for triglyceride removal have become saturated, or that corticoid treatment inhibits removal, as well as stimulating production of triglycerides. The latter seems least likely in view of recent data indicating that corticoid treatment increases postheparin lipolytic activity in man (13). Plasma cholesterol accumulation after Triton is also increased in corticoid-treated rats, but the rise is not significantly different from that of the controls and the data are omitted for simplicity. As before, the more striking change in triglyceride concentrations suggests that it is VLDL that accumulates in the plasma of corticoid-treated rats. Plasma glucose levels[2] were also determined in control (125 ± 6 mg/100 ml) and corticoid-treated animals (134 ± 8 mg/100 ml) (mean ± SD). This difference was not found to be statistically significant.

The increase in the amount of triglyceride present in the plasma after corticosteroid treatment both before and after Triton administration suggests that corticoid treatment increased the synthesis and/or release of VLDL. In order to more directly determine whether the liver was involved in this process, we examined the effect of corticoids on hepatic ultrastructure. Administration of methylprednisolone for 8 days caused an apparent increase in hepatocyte mitochondrial size (Figs. 2 and 3), as previously reported by Weiner et al. (21). Otherwise, the effect on general hepatocyte morphology was not striking (Figs. 2 and 3). Substantial numbers of rough endoplasmic reticulum membranes were apparent in all cells of the corticosteroid-treated group; hepatocytes from the corticosteroid-treated group did not show a special accumulation of neutral lipid as had been described previously (7); and tissue specimens

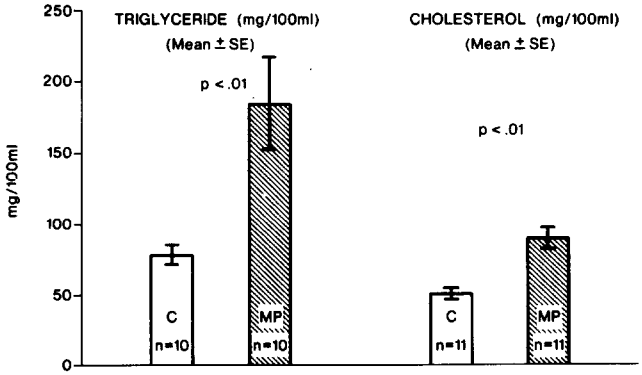

Fig. 1. Effect of methylprednisolone administration on the plasma triglyceride and cholesterol concentrations of normal rats. MP, methylpredisolone; C, control.

[2] Plasma glucose levels were measured with a Beckman glucose analyzer, Beckman Instruments, Fullerton, Calif.

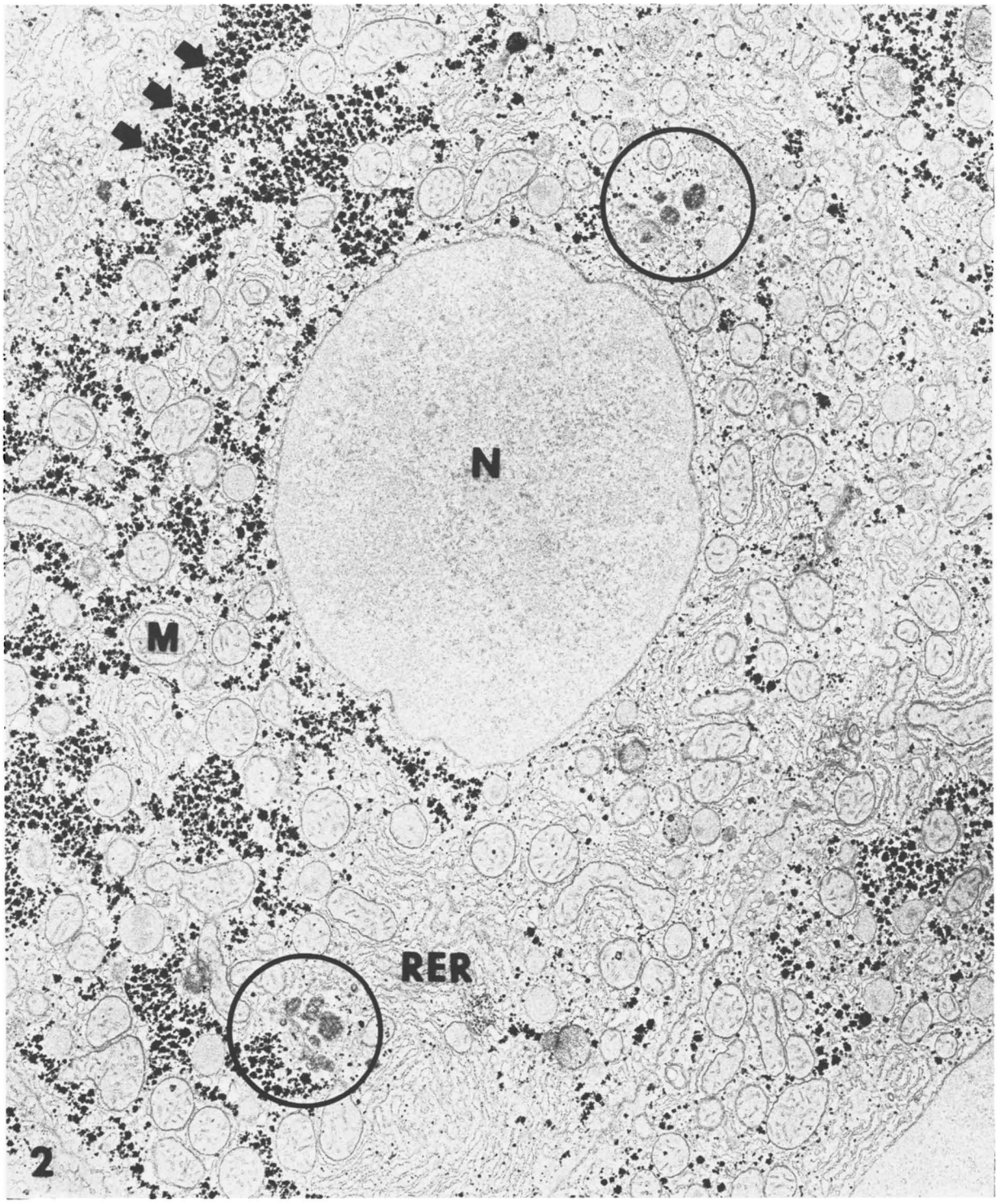

Fig. 2. Low magnification electron micrograph of a hepatocyte from a control rat (D29) showing nucleus (*N*) and normal distribution of mitochondria (*M*), rough endoplasmic reticulum (*RER*), glycogen deposits (*arrows*), and Golgi complexes (encircled). × 10,800.

postfixed in uranyl acetate revealed that the amount of VLDL associated with the smooth endoplasmic reticulum was comparable with that found in hepatocytes of the control rats.

In addition, corticoid administration did not appear to lead to an increase in the number of Golgi complexes per cell; however, there did seem to be an increase in the size of the individual Golgi complexes. Thus, in Fig. 3 the

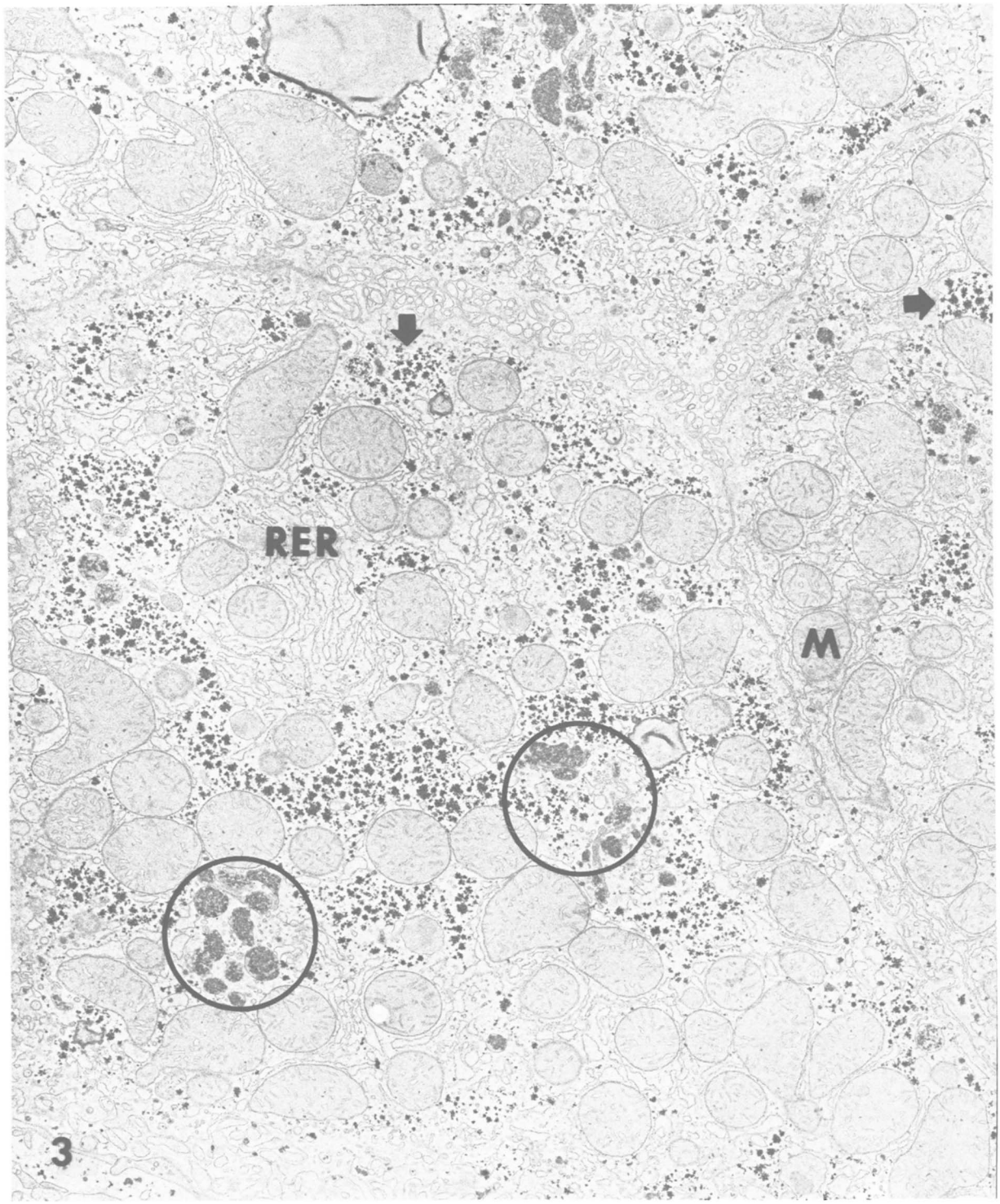

Fig. 3. Low magnification electron micrograph of a hepatocyte from a methylprednisolone-treated rat (D25) showing the distribution of mitochondria (*M*), rough endoplasmic reticulum (*RER*), glycogen deposits (*arrows*), and Golgi complexes (encircled). × 10,800.

Golgi complexes appear to be somewhat fuller and more rounded than the Golgi complexes of a hepatocyte from a control animal (Fig. 2). This change can be better appreciated in higher power electron micrographs that were used to determine the number and size of VLDL particles present in hepatocytes from normal (Fig. 4) and corticoid-treated animals (Fig. 5). A summary of these computations appears in Tables 2 and 3 and indicates that methylprednisolone produced a significant increase in both the number (Table 2) of VLDL particles within the Golgi

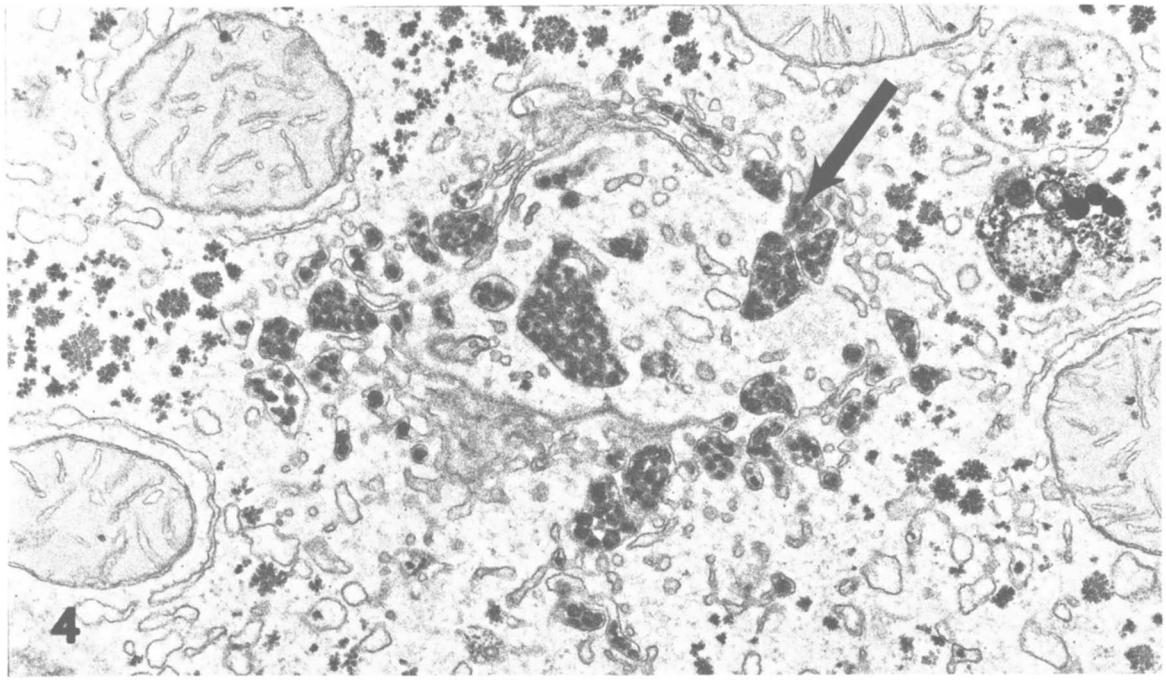

Fig. 4. Electron micrograph showing a typical large Golgi complex from a hepatocyte of a normal rat. The Golgi vesicles contain VLDL particles that average 50.0 nm in diameter (*arrow*). × 28,800.

complexes and the average diameter of the individual particles (Table 3).

The values in Table 3 for VLDL diameter are given in millimeters and represent the measured diameter of these particles magnified 28,000 times. These numbers can be converted mathematically to the actual dimensions of the particles in nanometers. When this is done, the VLDL particles from control animals that measure 1.5 mm are actually 53.5 nm (535 Å) in diameter and occupy a volume of 8.24×10^{-5} μm^3. These values are consistent with published measurements (22–27). The VLDL particles from corticosteroid-treated animals that measure 1.99

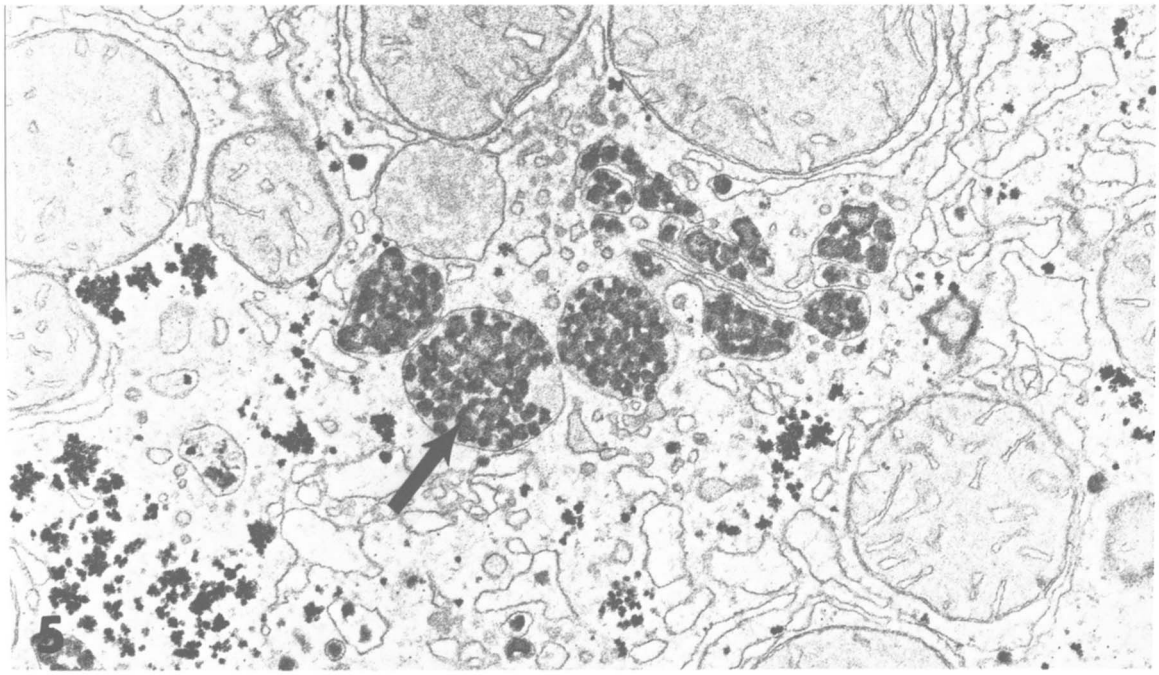

Fig. 5. Electron micrograph of a typical Golgi complex from a hepatocyte of a methylprednisolone-injected rat. The Golgi vesicles contain VLDL particles that average 70.0 nm in diameter (*arrow*). × 28,800.

TABLE 2. Effect of methylprednisolone on number of VLDL particles found in Golgi complexes of rat hepatocytes

Control		Methylprednisolone	
Rat		Rat	
D29	293 ± 22[a]	D24	523 ± 206
D30	326 ± 87	D25	460 ± 56
D31	362 ± 69	D26	559 ± 168
D32	353 ± 79	D27	545 ± 96
D33	333 ± 40	D28	551 ± 155
Mean ± SE = 333 ± 11		Mean ± SE = 528 ± 16	
$P < 0.0001$			

[a] Mean (± SD) number of Golgi VLDL particles in three hepatocytes per animal.

mm are 70.7 nm (707 Å) in diameter and occupy a volume of 18.5×10^{-5} μm^3. Thus, corticoid treatment results in a 33% increase in the diameter or a 120% increase in the volume of VLDL particles.

The estimation of VLDL size in tissue sections is complicated by the problem of tangential sectioning of the particles. In the case of the larger VLDL particles from homone-treated animals, the mean particle diameter, 70.7 nm, is larger than the thickness of the plastic section itself (50–60 nm), and one might therefore predict an increase in percentage of tangential cuts through the larger spheres. In an attempt to visualize the distribution of sizes of these particles, frequency distribution curves were prepared of all particles measured from Golgi vesicles (Fig. 6). As predicted from the differences in the mean diameters of the measured particles, the corticosteroid histogram curve is shifted to the right. There is, in addition, a slight "hump" of the corticosteroid curve in the region of the smaller VLDL particles that could be a reflection of an increased number of oblique slices through the larger (70.7 nm) VLDL particles. On the other hand, this "hump" could represent a population of "normal" sized 50-nm VLDL particles coexisting with larger particles in the hormone-treated animals.

Effect of adrenal corticosteroid treatment on hepatic and plasma lipoprotein metabolism in mice

The effect of methylprednisolone treatment on the lipid metabolism of mice was similar to that of rats. These results are summarized in Table 4 and indicate that corticoid treatment led to an elevation in plasma triglyceride and cholesterol concentrations, and this rise in plasma lipid levels was associated with an increase in the number and size of the VLDL particles present within the Golgi complexes of the hepatocyte.

DISCUSSION

The results of these studies indicate that administration of adrenal corticosteroids can cause hyperlipoproteinemia

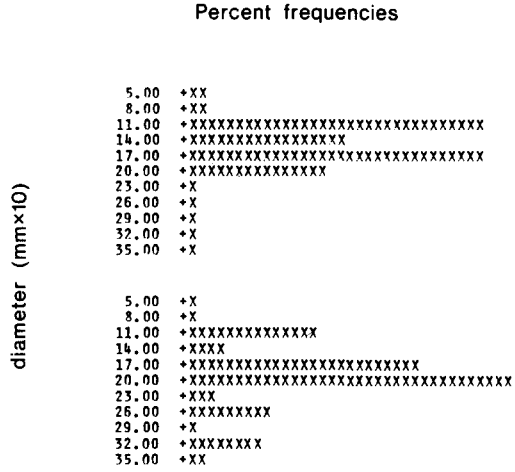

Fig. 6. Comparison of frequency distribution histograms representing 500 measured VLDL particles from hepatocytes of control rats (*top*) and 600 measured VLDL particles from hepatocytes of methylprednisolone-treated rats (*bottom*). Note that the histogram of VLDL particles from the hormone-treated animals is shifted to the right.

in normal rats and mice. Theoretically, hyperlipoproteinemia can result primarily from either an increase in the entry of lipids into the plasma or a decrease in lipid removal from this compartment. In the case of the hyperlipoproteinemia associated with the administration of adrenal cortical hormone, we feel that the current study supports the theory that increased entry of lipids into the plasma is the mechanism involved (13). This conclusion is based upon the combined evidence from the two major investigative efforts. The first line of evidence is based upon the results of the study with Triton WR 1339. This compound is considered to inhibit more than 90% of lipoprotein removal from the plasma (17, 18). If adrenal corticoids acted primarily to inhibit the removal of lipoproteins from plasma, the increments in plasma triglyceride and cholesterol levels after Triton WR 1339 administration should be similar in normal and corticoid-treated rats. If the increment in plasma lipid levels is greater in corticoid-treated rats, it supports the notion that increased lipoprotein entry, not decreased lipoprotein removal, is the cause of the hyperlipoproteinemia. Obviously this line of reasoning is valid only if the block of lipoprotein removal is equal in the experimental and control animals. The experimental evidence that Triton inhibits the removal of more than 90% of lipoproteins from the plasma compartment is excellent for normal rats (18). One must consider the possibility, however, that the effectiveness of the block may change in different experimental situations. For example, could corticoid treatment increase the block in lipoprotein removal from 90% to 100%, and could this account for the greater post-Triton increment in plasma triglyceride in the absence of a corticoid-induced increase in TG entry rate into plasma (Table 1)? The answer to this question is no. The TG entry rates in Table 1 are calcu-

TABLE 3. Effect of methylprednisolone on diameter of VLDL particles found in Golgi vesicles of rat hepatocytes[a]

Control			Methylprednisolone		
Rat	Number of VLDL Particles Measured/Animal	Diameter	Rat	Number of VLDL Particles Measured/Animal	Diameter
		mm[b]			*mm*[b]
D30	350	1.57 ± 0.28	D24	469	1.74 ± 0.44
D31	721	1.47 ± 0.45	D25	378	1.79 ± 0.60
D32	287	1.43 ± 0.30	D26	518	1.94 ± 0.66
D33	560	1.52 ± 0.42	D27	413	2.10 ± 0.45
			D28	532	2.37 ± 0.61
Mean diameter ± SE = 1.50 ± 0.03			Mean diameter ± SE = 1.99 ± 0.11		
$P > 0.01$					

[a] Measurements were derived from Golgi structures enlarged 28,000 times.
[b] Means ± SD.

lated on the basis of a 100% inhibition of lipoprotein removal. Thus, the values for the corticoid-treated rats, assuming 100% inhibition, are correct. However, if only a 90% inhibition exists in control rats there is a 10% leak, and the TG entry rate into plasma is being underestimated. However, a simple calculation can provide the necessary correction. Thus, the estimated TG entry rate of control rats in Table 1 (0.97 mg/min) is equal to the true entry rate minus the fractional leak of TG from plasma times the true entry rate. Solving for the true TG entry rate, and based upon a 90% inhibition of lipoprotein removal (or a 10% leak), it can be seen that the TG entry rate in normal animals is only increased from 0.97 mg/min to 1.08 mg/min. This value is still far less than the TG entry rate of 1.45 mg/min seen in corticoid-treated rats, even assuming that this discrepancy in degree of inhibition of lipoprotein removal exists. Similar calculations indicate that lipoprotein removal must be inhibited 100% in corticoid-treated rats as compared with 66% in normal rats in order to account for the differences in post-Triton TG entry rates solely on the basis of differences in degree of Triton blockade. This alternative seems most unlikely in light of the excellent review of this problem by Recknagel (18), who, after careful analysis of existing data, concluded that the block of lipoprotein removal was in excess of 90% in normal rats.

The second line of evidence is based upon the estimated increase in VLDL content of hepatocyte Golgi complexes of corticosteroid-treated rats and mice. VLDL particles are thought to be assembled within the endoplasmic reticulum. Many VLDL particles are subsequently transferred to the Golgi membranes, where, under normal circumstances, they are packaged into Golgi vesicles and transported through the hepatocyte to be emptied into the liver sinusoids. The period of time that individual VLDL particles spend within the Golgi vesicles associated with the Golgi complex prior to their transit through the cell has not yet been determined, and it is possible that this time varies under different physiological circumstances. In any event, the vesicles associated with the Golgi appear to act as temporary storage vessels for these particles, and at any moment in time their VLDL content can be measured. We have attempted to take advantage of this packaging of VLDL by estimating the number and volume of VLDL particles present in Golgi complexes of representative cells. When this was done the results indicated that adrenal corticoid treatment resulted in a statistically significant increase in both the number and size of the VLDL particles within the vesicles associated with the Golgi complexes of the hepatocytes. Thus, either more VLDL particles than normal are being synthesized by the hepatocytes or else removal of particles from the Golgi area is slowed down. Although these studies of hepatic ultrastructure cannot distinguish between these two events, it would seem that the first alternative, net increased synthesis of VLDL particles, offers the simplest explanation for both

TABLE 4. Effect of methylprednisolone on various aspects of hepatic and plasma lipoprotein metabolism in mice[a]

Group	Number of Mice	Triglycerides	Cholesterol	VLDL Particle Diameter in Golgi Vesicles	Number of VLDL Particles/Golgi
		mg/100 ml plasma		*mm*	
Control	7	71 ± 3	99 ± 4	1.49 ± 0.06	152 ± 10
MP-treated	7	117 ± 6[b]	123 ± 7[b]	1.78 ± 0.06[b]	226 ± 11[b]

[a] Results are means ± SE.
[b] MP-treated mice greater than control, $P < 0.01$.

the hyperlipoproteinemia and the incremental changes in plasma lipid levels that follow the administration of Triton WR 1339.

Although measurements of hepatocyte VLDL particle size and number indicate that corticoids increase hepatic content of Golgi VLDL, the significance of these results is dependent to a certain extent upon the reliability of the techniques used. In this regard, an effort was made in all aspects of this study to minimize the degree of subjectivity in both the sampling of cells and in the measurement of VLDL particles within cells. In the case of the measurement of VLDL particles this was accomplished by virtue of the fact that all estimates of both number and size of VLDL particles were performed without knowledge of the source of the tissue being examined. On the other hand, for technical reasons it was impossible to be equally objective in the selection of the cells to be analyzed. For example, to obtain cells that showed uniformly good preservation of VLDL, only those portal–midzonal areas of the liver lobule that appeared on an outside edge of the tissue block were chosen. Moreover, only those cells that were nucleated and that were complete within a single grid opening were chosen for analysis. The cells had to be the appropriate thinness as determined by the experience of the microscopist, and the Golgi of the cells had to contain well-stained, round VLDL particles. In the rat studies in which Golgi VLDL were counted directly at the microscope level, the first three cells that fulfilled the above criteria were selected, and the VLDL particles within all Golgi were counted. The relatively small variation in hepatocyte Golgi VLDL number among the five rats in each group, as seen in Table 2, suggests that this method of cell selection provided reasonably reproducible data. A greater degree of subjectivity was introduced in the estimates of VLDL particle size, in which only two Golgi complexes, estimated to be the largest from each of seven hepatocytes per animal, were used to measure the size of the VLDL particles. However, there is no reason to assume that measuring the size of VLDL particles in only the two largest Golgi would affect estimates of individual VLDL particle size, and the data in Tables 3 and 4 for rat and mice indicate that this approach also provided reasonably reproducible results. However, the use of only the two largest Golgi complexes to estimate the average number of VLDL particles within Golgi complexes in the mouse studies introduced a systematic error, insofar as the mean Golgi VLDL number (Table 4) is larger than would have been the case if the VLDL particles in all the hepatocyte Golgi complexes had been counted. However, even in this case, the degree of reproducibility was good, and the method demonstrated a significant difference between the control and treated groups. Therefore, for the reasons outlined, we feel the ultrastructural morphometric methods used in this study are reliable and provide supportive evidence that corticoid treatment results in an increase in VLDL within hepatocyte Golgi complexes.

It should be emphasized that the observed changes in lipoprotein concentration in plasma and liver were not accompanied by any evidence of fatty liver. The fact that corticoid administration has led to the development of fatty liver in other studies (7) is possibly related to the use of higher corticoid doses. The manner in which such a pathological reaction of the liver might affect the size and number of VLDL particles present in Golgi vesicles and ultimately the level of lipoprotein particles in the plasma is not clear. We consider this to be a problem worthy of investigation but only peripherally related to this current study.

It should also be pointed out that the effect of corticoid treatment on plasma lipid levels described in this paper is quite different from the cases described by Bagdade, Porte, and Bierman (28). In their patients corticoid treatment led to insulin deficiency, severe hyperglycemia, and ketoacidosis. None of these possible effects of corticoid treatment occurred in the rats and mice given corticoids in this study, and, indeed, hyperinsulinemia, not hypoinsulinemia, is the rule when corticoids are given to normal subjects (13, 29).

In conclusion, corticoid administration results in an elevation of plasma triglyceride and cholesterol levels, an increase in accumulation of triglycerides in plasma after lipoprotein removal is blocked by Triton, and an increase in the number and size of the Golgi VLDL particles present in the hepatocyte. We believe that these combined results strongly support the theory that hyperlipemia associated with adrenal corticoid administration is due to increased production of VLDL. On the other hand, the entire increment in VLDL lipoprotein production need not be due to increased synthesis and release of VLDL from the liver. It is now quite clear that intestinal cells can also manufacture VLDL lipoproteins (30–32). Corticoids may also stimulate VLDL production by the intestine, and it is possible that some of the increment in plasma lipid levels stems from increased VLDL synthesis and release by the intestine. We have begun to investigate this interesting possibility in our laboratory.

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REFERENCES

1. Adlersberg, D., S. R. Drachman, and L. E. Schaefer. 1951. Effects of cortisone and ACTH on serum lipids in animals:

possible relationship to experimental atherosclerosis. *Circulation.* **4:** 475. (Abstr.)
2. Bloom, B., and F. T. Pierce, Jr. 1952. Relationship of ACTH and cortisone to serum lipoproteins and atherosclerosis in humans. *Metabolism.* **1:** 155–162.
3. Migeon, C. J. 1952. Effect of cortisone on lipids of serum, liver and testes in intact and adrenalectomized rats. *Proc. Soc. Exp. Biol. Med.* **80:** 571–574.
4. DiLuzio, N. R., M. L. Shore, and D. B. Zilversmit. 1954. Effect of cortisone and deoxycorticosterone acetate on plasma lipids of adrenalectomized dogs. *Metabolism.* **3:** 424–432.
5. Skanse, B., W. von Studnitz, and N. Skoog. 1959. The effect of corticotrophin and cortisone on serum lipids and lipoproteins. *Acta Endocrinol.* **31:** 442–450.
6. Moran, T. J. 1962. Cortisone-induced alterations in lipid metabolism. *AMA Arch. Pathol.* **73:** 52–64.
7. Hill, R. B., Jr., and W. A. Droke. 1963. Production of fatty liver in the rat by cortisone. *Proc. Soc. Exp. Biol. Med.* **114:** 766–769.
8. Friedman, M., J. Van den Bosch, S. O. Byers, and S. St. George. 1965. Effects of cortisone on lipid and cholesterol metabolism in the rabbit and rat. *Amer. J. Physiol.* **208:** 94–105.
9. Hill, R. B., Jr., W. A. Droke, and A. P. Hays. 1965. Hepatic lipid metabolism in the cortisone-treated rat. *Exp. Mol. Pathol.* **4:** 320–327.
10. Klausner, H., and M. Heimberg. 1967. Effect of adrenalcortical hormones on release of triglycerides and glucose by liver. *Amer. J. Physiol.* **212:** 1236–1246.
11. Mahley, R. W., M. E. Gray, R. L. Hamilton, and V. S. LeQuire. 1968. Lipid transport in liver. II. Electron microscopic and biochemical studies of alterations in lipoprotein transport induced by cortisone in the rabbit. *Lab. Invest.* **19:** 358–369.
12. Kyner, J. L., R. I. Levy, J. S. Soeldner, R. E. Gleason, and D. S. Fredrickson. 1972. The short-term effect of cortisone acetate upon fasting triglyceride and cholesterol in normal subjects and offspring of diabetic couples. *Metabolism.* **21:** 329–336.
13. Stern, M. P., O. G. Kolterman, J. F. Fries, H. O. McDevitt, and G. M. Reaven. 1973. Adrenocortical steroid treatment of rheumatic diseases. Effects on lipid metabolism. *Arch. Intern. Med.* **132:** 97–101.
14. Rudman, D., and M. Di Girolamo. 1971. Effect of adrenal cortical steroids on lipid metabolism. *In* Human Adrenal Cortex. N. Christy, editor. Harper and Row, New York. 241–255.
15. Noble, R. P. 1968. Electrophoretic separation of plasma lipoproteins in agarose gel. *J. Lipid Res.* **9:** 693–700.
16. Otway, S., and D. S. Robinson. 1967. The effect of a nonionic detergent (Triton WR 1339) on the removal of triglyceride fatty acids from the blood of the rat. *J. Physiol.* (London). **190:** 309–319.
17. Otway, S., and D. S. Robinson. 1967. The use of a nonionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. *J. Physiol.* (London). **190:** 321–332.
18. Recknagel, R. O. 1967. Carbon tetrachloride hepatotoxicity. *Pharmacol. Rev.* **19:** 145–208.
19. N-78 "Triglycerides" method *in* AutoAnalyzer Manual, Technicon Instruments, Chauncey, N.Y. 1964.
20. N-24a "Total cholesterol in serum" method *in* AutoAnalyzer Manual, Technicon Instruments, Chauncey, N.Y. 1964.
21. Weiner, J., A. V. Loud, D. V. Kimberg, and D. Spiro. 1968. A quantitative description of cortisone-induced alterations in the ultrastructure of rat liver parenchymal cells. *J. Cell Biol.* **37:** 47–61.
22. Claude, A. 1970. Growth and differentiation of cytoplasmic membranes in the course of lipoprotein granule synthesis in the hepatic cell. I. Elaboration of elements of the Golgi complex. *J. Cell Biol.* **47:** 745–766.
23. Parks, H. F. 1967. An experimental study of microscopic and submicroscopic lipid inclusions in hepatic cells of the mouse. *Amer. J. Anat.* **120:** 253–280.
24. Hamilton, R. L., D. M. Regen, M. E. Gray, and V. S. LeQuire. 1967. Lipid transport in liver. I. Electron microscopic identification of very low density lipoproteins in perfused rat liver. *Lab. Invest.* **16:** 305–319.
25. Ruderman, N. B., A. L. Jones, R. M. Kraus, and E. Shafrir. 1971. A biochemical and morphologic study of very low density lipoproteins in carbohydrate-induced hypertriglyceridemia. *J. Clin. Invest.* **50:** 1355–1368.
26. Mahley, R. W., R. L. Hamilton, and V. S. LeQuire. 1969. Characterization of lipoprotein particles isolated from the Golgi apparatus of rat liver. *J. Lipid Res.* **10:** 433–439.
27. Jones, A. L., N. B. Ruderman, and M. G. Herrera. 1967. Electron microscopic and biochemical study of lipoprotein synthesis in the isolated perfused rat liver. *J. Lipid Res.* **8:** 429–446.
28. Bagdade, J. D., D. Porte, Jr., and E. L. Bierman. 1970. Steroid-induced lipemia. A complication of high-dosage corticosteroid therapy. *Arch. Intern. Med.* **125:** 129–134.
29. Perley, M., and D. M. Kipnis. 1966. Effect of glucocorticoids on plasma insulin. *N. Engl. J. Med.* **274:** 1237–1241.
30. Mahley, R. W., B. D. Bennett, D. J. Morré, M. E. Gray, W. Thistlethwaite, and V. S. LeQuire. 1971. Lipoproteins associated with the Golgi apparatus isolated from epithelial cells of rat small intestine. *Lab. Invest.* **25:** 435–444.
31. Ockner, R. K., F. B. Hughes, and K. J. Isselbacher. 1969. Very low density lipoproteins in intestinal lymph: role in triglyceride and cholesterol transport during fat absorption. *J. Clin. Invest.* **48:** 2367–2373.
32. Windmueller, H. G., F. T. Lindgren, W. J. Lossow, and R. I. Levy. 1970. On the nature of circulating lipoproteins of intestinal origin in the rat. *Biochim. Biophys. Acta.* **202:** 507–516.