Hypersecretion of VLDL, but not HDL, by hepatocytes from the JCR:LA-corpulent rat

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Abstract The JCR:LA-corpulent male rat, when homozygous for the cp gene (cp/cp) is hyperlipidemic and prone to atherosclerosis. Both male and female cp/cp rats have markedly elevated serum levels of triacylglycerols and phospholipids [Dolphin, P. J. et al. 1987. *Biochim. Biophys. Acta.* **919:** 140–148]. In the present study, monolayer cultures of hepatocytes were prepared from male and female, corpulent and lean, rats. There was a marked hypersecretion of all very low density lipoprotein (VLDL) lipid and apoprotein components from corpulent-derived cells. The increased secretion most likely accounts for the increased levels of VLDL lipids and apoproteins previously observed in serum. In contrast, there was no difference between the corpulent and lean hepatocytes in their secretion of high density lipoprotein (HDL) lipids and apoproteins. The difference in triacylglycerol secretion between the lean and corpulent cells was sustained even when the cells were cultured for 24, 48, and 72 h prior to the experiment, by which time the hormonal differences between the corpulent and lean animals would have been largely eliminated. The magnitude of the difference in triacylglycerol secretion did not diminish with increasing time in culture. The biochemical basis responsible for the hypersecretion of VLDL has not yet been established. However, preliminary results suggest that there is an inherent difference in glycerolipid metabolism in the two types of hepatocytes. **—Vance, J. E., and J. C. Russell.** Hypersecretion of VLDL, but not HDL, by hepatocytes from the JCR:LA-corpulent rat. *J. Lipid Res.* 1990. **31:** 1491–1501.

Supplementary key words hyperlipidemia • very low density lipoprotein • high density lipoprotein • lipoprotein secretion • triacylglycerol

The JCR:LA-corpulent rat is an obese, hyperlipidemic, hyperinsulinemic strain in which corpulent males spontaneously develop vascular and myocardial lesions while lean and female animals do not (1). Thus, this strain of rat may be a useful model for studying the process of atherogenesis. Only the homozygotes for the recessive gene (cp/cp) are corpulent and hyperlipidemic, whereas homozygous normal (+ / +) or heterozygous (+ / −) rats are not (1). The corpulent rats have markedly elevated levels of serum VLDL and HDL (2). Moreover, the total serum cholesterol level (esterified and unesterified) is elevated 2-fold and the TG level is elevated by at least 40-fold, in the cp/cp compared with the lean (+ / +) rat serum (2). Interestingly, the female corpulent cp/cp animals (which are not especially susceptible to atherosclerosis) have a 2-fold greater content of TG in their serum and in their VLDL than do their male counterparts (2). Similarly, the phospholipid content of the serum VLDL and HDL fractions from corpulent males was elevated 25- and 3-fold, respectively, compared with the lean males (2).

The serum apoprotein levels have also been compared in the corpulent and lean rats (2). The levels of apoA-I and apoE were significantly higher (2.9- and 1.8-fold, respectively) in the cp/cp compared to the control rat serum (2). Surprisingly, however, the level of apoB in the male cp/cp rat serum was not significantly higher than in the + / + animals (2).

The hypertriglyceridemia and hypercholesterolemia observed in the serum of the corpulent rats may have been the result of an increased rate of lipoprotein secretion and/or a decreased rate of lipoprotein removal from the circulation. Preliminary studies on the rates of lipid clearance and the apparent hepatic secretion rate, using Triton WR1339 as an inhibitor of lipoprotein lipase (3), suggested that the rate of secretion of TG was markedly (5- to 6-fold) higher in the corpulent males than in the lean males, and even higher in the corpulent females, in concert with the differences in their serum TG levels. However, such studies of lipoprotein secretion rates in intact animals are complicated by the complex metabolism of lipoproteins that occurs in the circulation and by the secretion of lipoproteins by both the liver and the intestine. Therefore, the present study was undertaken as a direct comparison of the rates of lipoprotein secretion by male and female, cp/cp and lean, rats using monolayer cultures of isolated hepatocytes. In this model system the

Abbreviations: TG, triacylglycerol; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdSer, phosphatidylserine; PtdIns, phosphatidylinositol; apo, apolipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

process of lipoprotein secretion is clearly dissociated from lipoprotein uptake, since there is apparently very little reuptake of newly secreted VLDL by cultured hepatocytes (4). In addition, primary rat hepatocytes can be cultured in a medium of defined composition, independent of exogenous hormonal variations. The experiments demonstrated that in hepatocytes cultured from the cp/cp rats there was a marked hypersecretion of all VLDL components, both lipids and apoproteins, which could explain the increased levels of VLDL lipids and apoproteins previously observed in the serum (2). In contrast, there was no difference in secretion of the HDL lipids and apoproteins by hepatocytes from the two types of animals.

MATERIALS AND METHODS

Materials

L-[4,5-^{3}H]leucine (69 Ci/mmol) and [2-^{3}H]glycerol (1 Ci/mmol) were purchased from Amersham Canada, Oakville, Ontario, Canada. Earle's minimum essential medium was obtained from Gibco Laboratories, Grand Island, NY, and fetal bovine serum was from BDH Chemicals, Ltd. The Primaria culture dishes (60 mm) were from Becton Dickson, Oxnard, CA. Phospholipid standards were purchased from Avanti Polar Lipids, Birmingham, AL, and triacylglycerol standard (triolein) was from Sigma, St. Louis, MO. The silica gel G 60 thin-layer chromatography plates (0.25 mm thickness) and the high performance thin-layer chromatography plates (silica gel 60, 10 × 20 cm) were obtained from BDH Chemicals Ltd. Cab-O-Sil (fumed silica) was from Sigma. EN^{3}HANCE was purchased from New England Nuclear. Soluene 350 and Hionic-Fluor scintillant were from Packard Instrument Company, Downer's Grove, IL. All reagents for polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories.

Animals and preparation of hepatocytes

The JCR:LA-corpulent rat strain is one of five strains incorporating the mutant corpulent (cp) gene orginally isolated by Koletsky (5). While it was originally described as the LA/N-corpulent strain, it has recently been designated as JCR:LA-corpulent to distinguish its significant genetic differences from the fully congenic LA/N-cp rat. The strain has previously been described (1–3, 6). Rats are bred from stock known to be heterozygous for the cp gene (cp/+) or homozygous normal (+/+). Breeding of cp/+ animals yields 25% cp/cp corpulent rats and 75% lean (either cp/+ or +/+). The lean rats from such breeding (i.e., cp/+ and +/+) are not distinguishable and are designated as +/?.

Primary rat hepatocytes were isolated from livers of young rats (approximately 6 to 8 weeks old) by the collagenase perfusion technique as described previously (4, 7). The cells (3 × 10^6/60-mm dish) were plated in Earle's minimum essential medium containing 17% fetal bovine serum, and allowed to adhere overnight to the dishes at 37°C in a 5% CO_2 atmosphere. For each experiment, the medium was removed the morning after the cells were plated and the cells were washed with Earle's minimum essential medium. The experimental medium, without serum, was then added. Cell viability was checked by Trypan blue exclusion and by leakage of lactate dehydrogenase (8). In either case, the cell viability was >90% for hepatocytes from both corpulent and lean rat livers. The protein content of a single 60-mm dish (3 × 10^6 cells) of corpulent or lean hepatocytes was the same. No differences in size or appearance of the two types of cells were observed in the phase contract microscope.

Isolation of lipoproteins

The culture medium from eight dishes of cells was combined (16 ml) and centrifuged at 10,000 *g* for 10 min to remove any cell debris. Lipoproteins from the medium were fractionated according to density by single-spin ultracentrifugation on a salt gradient (7) for 42 h at 100,000 *g* in a Beckman Ti 70 rotor. Fractions corresponding to the following densities were collected: VLDL, d < 1.02 g/ml; LDL, d between 1.02 and 1.06 g/ml; HDL, d between 1.06 and 1.18 g/ml; bottom fraction, d > 1.18 g/ml. The lipoproteins in each fraction were concentrated by the addition of Cab-O-Sil (fumed silica) (7). The Cab-O-Sil, containing the bound lipoproteins, was pelleted by centrifugation at 12,000 *g* for 10 min and the lipids and/or apoproteins were extracted from the Cab-O-Sil, as described below.

Isolation and analysis of lipids from lipoproteins and hepatocytes

Lipids were extracted from cells with chloroform–methanol 2:1 (v/v) according to the method of Folch, Lees, and Sloane Stanley (9). Lipids were extracted from the Cab-O-Sil-bound lipoproteins, from either total or density-fractionated medium, by the addition of 5 ml of chloroform–methanol 2:1 (v/v). The Cab-O-Sil was pelleted by centrifugation at 10,000 *g* for 15 min and the lipid extract was removed to a clean tube containing 1.6 ml water. The sample was vortexed, then centrifuged for 10 min at 2,000 *g* to allow separation of the phases. The lower phase was washed twice with 3.2 ml methanol–water 1:1 (v/v). Thin-layer chromatography of cellular, medium, or lipoprotein lipids on silica gel G plates in the solvent system chloroform–methanol–acetic acid–formic acid–water 70:30:12:4:2 (v/v) permitted separation of the individual phospholipids PtdCho, PtdEtn, PtdSer, and PtdIns, which were identified by comparison with standards. The lipids were visualized under ultraviolet light after the plate had been sprayed with Primulin (10). The mass of the individual phospholipids was measured by phosphorus analysis according to the method of Rouser,

Siakotos, and Fleischer (11). In experiments in which labeled phospholipids were isolated, the phospholipid bands on the silica gel were scraped into scintillation vials and radioactivity was counted directly.

Triacylglycerols were separated from the total lipid extracts of cells, medium, or lipoproteins by thin-layer chromatography in hexane–diisopropyl ether–acetic acid 65:35:2 (v/v), using triolein as a standard. The mass of TG was determined by treatment with alkaline hydroxylamine (12). In experiments in which radioactive incorporation into TG was measured, the band containing TG was scraped from the plate directly into a scintillation vial and counted for radioactivity. In some experiments, both TG and phospholipids were simultaneously separated on a single plate using first chloroform–methanol–acetic acid–formic acid–water 70:30:12:4:2 (v/v) run halfway up the plate. The plate was dried, then run to the top in hexane–diisopropyl ether–acetic acid 65:35:2 (v/v). For high performance thin-layer chromatography (13), an aliquot of the total lipid extract was chromatographed by development of the plate half-way in chloroform–methanol–acetic acid–formic acid–water 70:30:12:4:2 (v/v). The plate was dried and the neutral lipids were separated by development in hexane–diisopropyl ether–acetic acid 65:35:2 (v/v) to the top of the plate. For visualization of the lipids the high performance plate was dipped in a solution of 3% (w/v) cupric acetate and phosphoric acid (8%, v/v) and heated at 180°C for 15 min (13).

Analysis of apoproteins from lipoproteins

Hepatocytes were incubated with 15 μCi/dish of [4,5-^{3}H]leucine for 5 h and the VLDL, LDL, HDL, and the bottom fraction were isolated, as above. Apoproteins were solubilized from the Cab-O-Sil pellet by the addition of 1 ml of buffer containing 2% sodium dodecyl sulfate and 6 M urea (7). Aliquots, plus unlabeled carrier lipoproteins isolated from rat serum by sequential flotation (14), were applied to a 3–15% polyacrylamide gradient gel containing 0.1% sodium dodecyl sulfate. The apoproteins were identified by comparison with standard apoproteins from rat serum. The gels were stained with Coomassie blue and the bands corresponding to apoB_H, apoB_L, apoE, apoC, apoA-I, and albumin were cut from the gels with a razor blade. The gel slices were incubated with 1 ml of Soluene 350 overnight and the amount of radioactivity was determined after incubation of the samples with 10 ml Hionic-Fluor scintillant for 3 days.

In experiments in which the mass of secreted apoproteins was compared in the medium from cp/cp and control hepatocytes, the polyacrylamide gel was run exactly as above, but without the addition of carrier standard lipoproteins. The apoproteins were visualized by staining with Coomassie blue (15) and compared with standard rat serum lipoproteins (14). The relative intensities of the stained protein bands were measured using a Camag density scanner at 550 nm. The area under each peak was integrated with a Camag SP4290 integrator.

Incorporation of [4,5-^{3}H]leucine into cellular proteins

One dish of hepatocytes was incubated for various times up to 24 h with 5 μCi of [4,5-^{3}H]leucine. The cells were harvested, then disrupted by vigorous vortexing in 0.5 ml water. Trichloroacetic acid (0.5 ml of 20% solution) was added and the precipitated proteins were pelleted by centrifugation at 2,000 *g* for 5 min The pellet was washed 3 times with 1 ml of ice-cold 5% trichloroacetic acid and the proteins were solubilized by addition of 0.8 ml of 0.1 M sodium hydroxide. The mixture was neutralized with 0.2 ml of 0.5 M HCl containing 0.12 M Tris, and the samples were counted for radioactivity.

Incorporation of [2-^{3}H]glycerol into glycerolipids of hepatocytes and culture medium

Monolayer cultures of hepatocytes were incubated with 5 μCi/dish of [2-^{3}H]glycerol. After various time intervals up to 24 h lipids were extracted from two dishes of medium or cells. The incorporation of ^{3}H into TG was measured after thin-layer chromatography of the glycerolipids on silica gel G plates, as described above.

Other methods

Protein was determined according to the method of Lowry et al. (16) using bovine serum albumin as a standard. Cell viability was assessed by both Trypan blue exclusion and by measurement of leakage of lactate dehydrogenase into the culture medium (8).

RESULTS

Lipid content of hepatocytes and rates of lipid secretion

Hepatocytes from lean and corpulent male and female rats were plated in serum-containing medium overnight; the cells were washed and the medium was replaced with Earle's minimum essential medium without serum. Cell viability was greater than 90% as judged by Trypan blue exclusion and leakage of lactate dehydrogenase into the culture medium.

The glycerolipid content of the hepatocytes in given in **Table 1**. There were no significant differences in the phospholipid content of the hepatocytes from the corpulent and lean rats, either males or females. For the males, the amounts of TG in the cp/cp and +/+ cells were not significantly different, whereas for females the TG content of the cp/cp cells was 1.9-fold higher than that of lean cells ($0.005 < P \leq 0.01$). There was also a 2.1-fold higher TG content in the cells from the female, compared with the male, cp/cp rats ($0.0005 < P < 0.005$).

TABLE 1. Glycerolipid composition of hepatocytes from male and female corpulent and lean rats

Lipid Fraction	Male Rats		Female Rats	
	+/+	cp/cp	+/?	cp/cp
	nmol/mg cell protein			
TG	12.6 ± 6.2	17.5 ± 7.0	18.7 ± 3.4	36.1 ± 8.9
PtdCho	61.4 ± 5.5	61.7 ± 7.7	68.5 ± 18.1	65.1 ± 19.0
PtdEtn	23.4 ± 2.3	24.3 ± 4.2	25.6 ± 6.5	28.9 ± 4.1
PtdSer	1.9 ± 0.6	1.6 ± 0.7	1.9 ± 0.3	1.6 ± 0.2
PtdIns	2.2 ± 0.4	2.2 ± 0.4	2.6 ± 0.7	3.1 ± 0.8

Monolayer cultures of hepatocytes were prepared from male and female, corpulent and lean, rats. Total lipids were extracted from the cells and separated into glycerolipid classes by thin-layer chromatography. The TG values are means ± SD of triplicate determinations from each of five individual experiments. The other lipids were measured from triplicate determinations of three individual experiments. There is no statistical difference between the TG content of male corpulent versus lean cells ($0.1 < P \leq 0.375$), nor between male and female lean cells ($0.05 < P \leq 0.1$). However, the TG content of female cp/cp cells is significantly higher than either lean female cells ($0.005 < P \leq 0.01$) or male cp/cp cells ($0.0005 < P \leq 0.005$). The statistics are calculated according to the unpaired *t*-test.

The amount of TG in the culture medium of cp/cp and lean control cells was compared after various time intervals up to 24 h (**Fig. 1A**, males; Fig. 1B, females). The rate of TG secretion from the hepatocytes of both male and female corpulent rats was 3- to 4-fold higher than from hepatocytes of the lean rats (Figs. 1A and 1B, **Table 2**). The rate of TG secretion in the female cp/cp and lean hepatocytes, however, was approximately 1.5-fold higher than in the male cp/cp and +/+ hepatocytes, respectively. In concert with the increased TG secretion by the corpulent hepatocytes there was a corresponding, though smaller, increase in the rate of secretion of PtdCho (Table 2 and Figs. 1C and D). Thus, the hyperlipidemia observed in the blood of the corpulent rats (2) and the increased rate of TG and phospholipid secretion observed in the intact animals (3) were also evident in the medium of isolated hepatocytes cultured from these rats.

Most likely, after the hepatocytes have been in culture for 20 h, residual hormonal differences between the cells derived from the lean and corpulent rats will have been largely abolished so that any differences in lipoprotein content cannot be attributed to hormonal differences between the two types of cells. That this was true was confirmed by culturing hepatocytes from both male and female, corpulent and lean, rats for 24, 48, or 72 h prior to measurement of the amount of TG secreted into the medium over an additional 6-h period. At all times examined, the difference in TG secretion between the corpulent and lean cells was sustained and the magnitude of the difference did not diminish with increasing time in culture (data not shown).

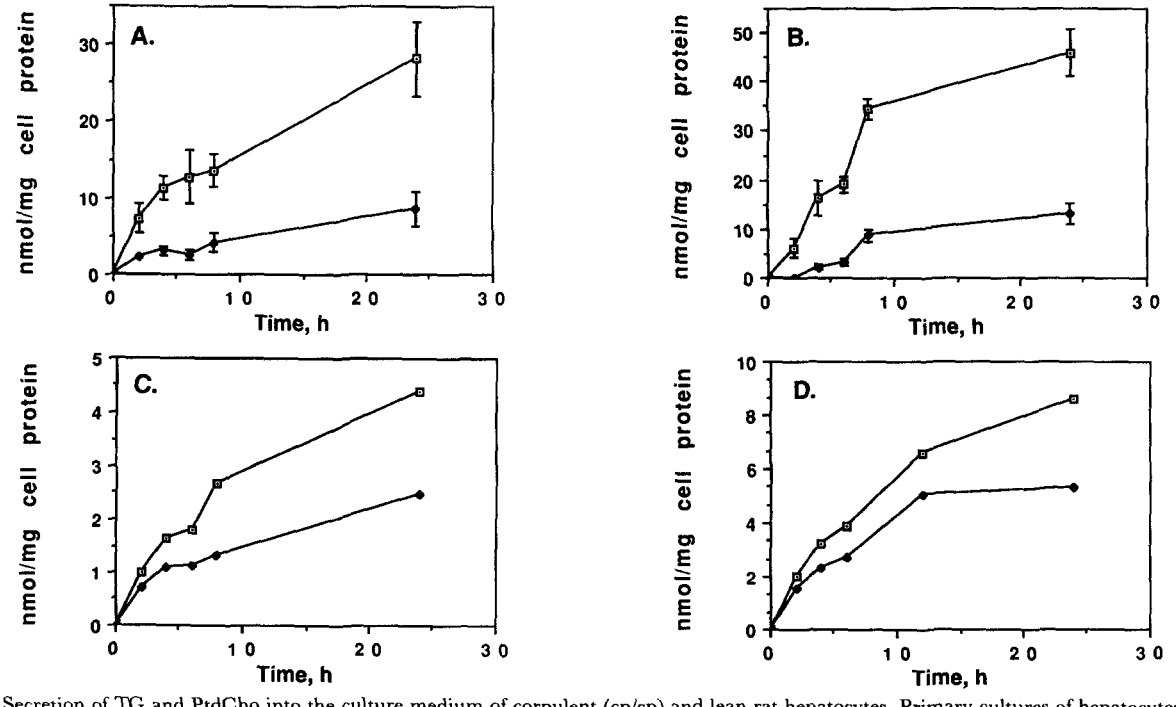

Fig. 1. Secretion of TG and PtdCho into the culture medium of corpulent (cp/cp) and lean rat hepatocytes. Primary cultures of hepatocytes from corpulent (□) and lean (♦), male (panel A and C) and female (panels B and D), rats were plated overnight in serum-containing medium. The cells were washed and incubated for various times, up to 24 h, in serum-free medium. Lipoproteins in the culture medium were concentrated by the addition of Cab-O-Sil and TG and PtdCho were isolated by thin-layer chromatography. The data for TG are averages ± SD of three experiments. The data for PtdCho are the averages of two similar experiments. Panels A and B, TG; panels C and D, PtdCho.

TABLE 2. Rate of glycerolipid secretion by hepatocytes cultured from male and female corpulent (cp/cp) and lean (+/+ or +/?) rats

Animal	Rate of Secretion	
	TG	PtdCho
	nmol/h per mg cell protein	
Male cp/cp	1.94 ± 0.24	0.34
Male +/+	0.43 ± 0.11	0.16
Female cp/cp	2.86 ± 1.26	0.55
Female +/?	0.73 ± 0.34	0.41

Hepatocytes were prepared from corpulent and lean, male and female, rats and the amounts of TG and PtdCho were measured in the culture medium of eight dishes of cells combined after various time intervals, as described for Fig. 1. Rates of secretion of TG and PtdCho were calculated over the first 12 h of incubation. For TG, values are averages ± SD of three similar experiments. For PtdCho, the rates are averages of two similar experiments, for which the individual values did not differ from the means by greater than 25%.

Incorporation of [2-^{3}H]glycerol into culture medium and cellular glycerolipids of corpulent (cp/cp) and lean (+/+) rat hepatocytes

The secretion of TG was also examined by incubation of hepatocytes from male corpulent and lean rats with [2-^{3}H]glycerol. After various times of incubation the lipids were extracted from cells and culture medium, and TG was purified by thin-layer chromatography. In agreement with the measurement of TG mass (Fig. 1A), the secretion of [^{3}H]glycerol-labeled TG was approximately 3-fold higher from the male corpulent hepatocytes than from the lean cells (**Fig. 2B**). Interestingly, the incorporation of [^{3}H]glycerol into cellular TG of the cp/cp hepatocytes for the first 4 to 8 h was markedly higher than in the lean hepatocytes (Fig. 2A). However, after 8 h the level of [^{3}H]TG was the same in both types of cells. Thus, the initial rate of incorporation of [^{3}H]glycerol into TG was much greater in the corpulent cells. On the other hand, the rate of disappearance of label from these cells was much faster than from the lean hepatocytes. In the hepatocytes from both corpulent and lean rats, the loss of radiolabeled cellular TG that occurred between 2 h and 24 h (Fig. 2A) could be almost completely accounted for by the accumulation of labeled TG in the culture medium (Fig. 2B). In the TG of the corpulent cells approximately 175,000 dpm/mg cell protein was lost between 2 h and 24 h. During the same time period approximately 140,000 dpm/mg cell protein was present in the medium. In the hepatocytes from the lean rats, 58,700 dpm/mg cell protein accumulated in TG in the medium, whereas 44,400 dpm/mg cell protein was lost from the cellular TG. The extra TG apparently synthesized in the hepatocytes from the corpulent, compared to the lean, rats did not accumulate significantly in the cells (Table 1) but was secreted into the medium.

Secretion of lipids into lipoprotein fractions

The culture medium from hepatocytes of cp/cp and lean, male and female, rats was fractionated on the basis of density, by single-spin ultracentrifugation, into VLDL, LDL, HDL, and a fraction of $d > 1.18$ g/ml (7). The high performance thin-layer chromatography lipid profiles (13) of the lipoprotein fractions are shown in **Figs. 3A and 3B**. The most striking difference between the lipoproteins from corpulent and control cells was that the concentration of all lipids (e.g., TG, PtdCho, PtdEtn, PtdIns, cholesterol, and sphingomyelin) was greatly elevated in the VLDL fraction from the corpulent, compared to the lean, hepatocytes. Similarly, in the LDL fraction, the concentration of all lipids was higher from the corpulent, than from the lean, hepatocytes (Figs. 3A and B). These data are in agreement with the hyperlipidemia in the serum of the corpulent rats (2). In contrast, in the HDL and bottom fractions ($d > 1.18$ g/ml) the differences in the amounts of lipids between the lipoproteins from corpulent

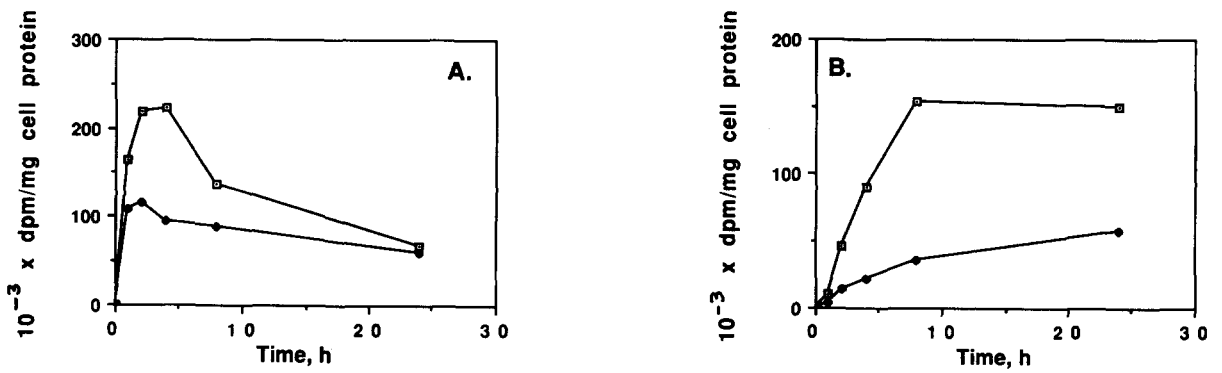

Fig. 2. Incorporation of [2-^{3}H]glycerol into TG of cells and medium from corpulent and lean male rat hepatocytes. Monolayer cultures of hepatocytes were incubated for various times, up to 24 h, with 5 μCi/dish of [2-^{3}H]glycerol. Lipids were extracted from cells and culture medium, and TG was isolated by thin-layer chromatography. Panel A, cellular TG from lean (♦) and corpulent (□) hepatocytes. Panel B, TG in the medium from lean (♦) and corpulent (□) hepatocytes. The data are mean values of two similar experiments for which the individual values did not differ from the mean values by more than 20%.

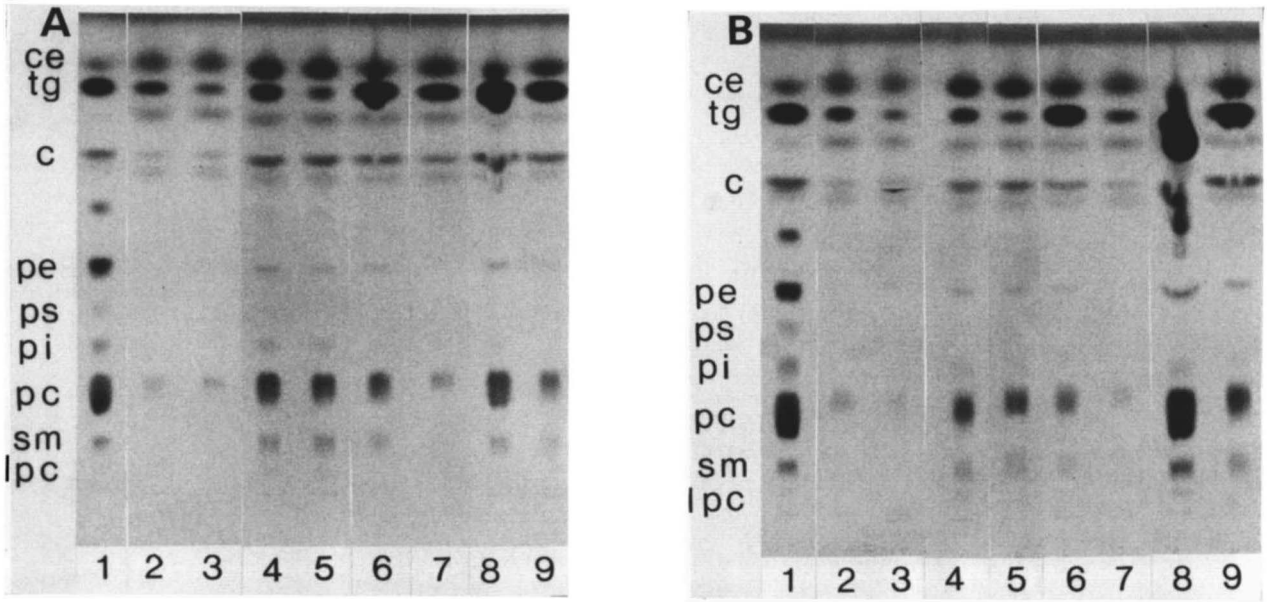

Fig. 3. High performance thin-layer chromatography of total lipids secreted into lipoproteins by male and female rat hepatocytes. Hepatocytes were cultured from male (panel A) and female (panel B) corpulent and lean rats. After 5 h, the culture medium was removed and the lipoprotein classes VLDL, LDL, HDL and a bottom fraction of density > 1.18 g/ml were separated on the basis of density by single-spin ultracentrifugation of the medium from eight dishes combined. The lipoproteins were concentrated by the addition of Cab-O-Sil and lipids were extracted. An aliquot equivalent to the lipid from two dishes of cells was used for VLDL, and from four dishes of cells for the LDL, HDL, and bottom fractions. The lipids were separated by high performance thin-layer chromatography and visualized by heating the plates after they had been dipped in a solution containing cupric acetate (3%) and phosphoric acid (8%). Abbreviations are: lpc, lysophosphatidylcholine; sm, sphingomyelin; pc, phosphatidylcholine; pi, phosphatidylinositol; ps, phosphatidylserine; pe, phosphatidylethanolamine; c, cholesterol; tg, triacylglycerol; ce, cholesteryl ester. The numbers along the base refer to the following samples: 1, rat liver lipid standards; 2 and 3, fraction of density > 1.18 g/ml; 4 and 5, HDL; 6 and 7, LDL; 8 and 9, VLDL. Lanes 2, 4, 6, 8 are from corpulent (cp/cp) hepatocytes and lanes 3, 5, 7, 9 are from lean (+/+ or +/?) hepatocytes.

and lean cells were insignificant, except for a slightly larger amount of TG in the samples from the cp/cp hepatocytes. Similar results were obtained for hepatocytes from the male (Fig. 3A) and female (Fig. 3B) rats. Thus, cp/cp hepatocytes secreted significantly larger amounts of lipids into the VLDL, but not the HDL, fraction than did the control hepatocytes.

Secretion of apoproteins into lipoprotein fractions

Secretion of the individual apoproteins of the lipoproteins from hepatocytes of lean and corpulent, male and female, rats was examined. The masses of apoproteins secreted by male and female cp/cp and control hepatocytes into VLDL and HDL were compared by polyacrylamide gel electrophoresis (**Fig. 4**). The bands corresponding to $apoB_H$, $apoB_L$, and apoE were scanned by a densitometer and the areas were integrated. The values for the integrated areas are given in the legend to Fig. 4. The intensities of the bands corresponding to VLDL apoproteins, $apoB_H$, $apoB_L$, and apoE were higher for the cp/cp cells than for the lean cells, for both male and female rats. In contrast, the mass of apoA-I, apoE, and albumin secreted into the HDL fraction by the corpulent and lean cells was very similar (Fig. 4). Surprisingly, in light of the higher TG secretion by hepatocytes from female compared with male rats (Table 2, Fig. 1), the amounts of VLDL apoproteins from the female cells were lower than from the males (Fig. 4). This result was confirmed in the experiments on [^{3}H]leucine labeling of the secreted apoproteins (see Fig. 5 compared with Fig. 6).

In other experiments on apoprotein secretion, hepatocytes were incubated with [4,5-^{3}H]leucine for 5 h. From the male rat hepatocytes, the amount of ^{3}H-labeled $apoB_H$, $apoB_L$, apoE, and apoC secreted into VLDL was 3.4-, 5.3-, 6.9-, and 3.8-fold, respectively, higher from the cp/cp, than from the control, hepatocytes (**Fig. 5A**). On the contrary, in the HDL fraction (Fig. 5C), there was no significant difference in secretion of labeled apoA-I, apoE, or apoC from the two types of cells. Similarly, for female rats the secretion of VLDL apoproteins B_H, B_L, E, and C from the corpulent hepatocytes was 2.7-, 2.9-, 4.6-, and 8.5-fold higher, respectively, than from the control hepatocytes (**Fig. 6A**), whereas in the HDL fraction there were only minimal differences in the apoproteins from the cp/cp and control cells (Fig. 6C). Only small amounts of radioactive apoproteins were present in the fraction of the LDL density range (Figs. 5B and 6B) in agreement with the low level of LDL in rat serum, but generally the trend was the same as for the VLDL fraction.

The hypersecretion of VLDL apoproteins was not due

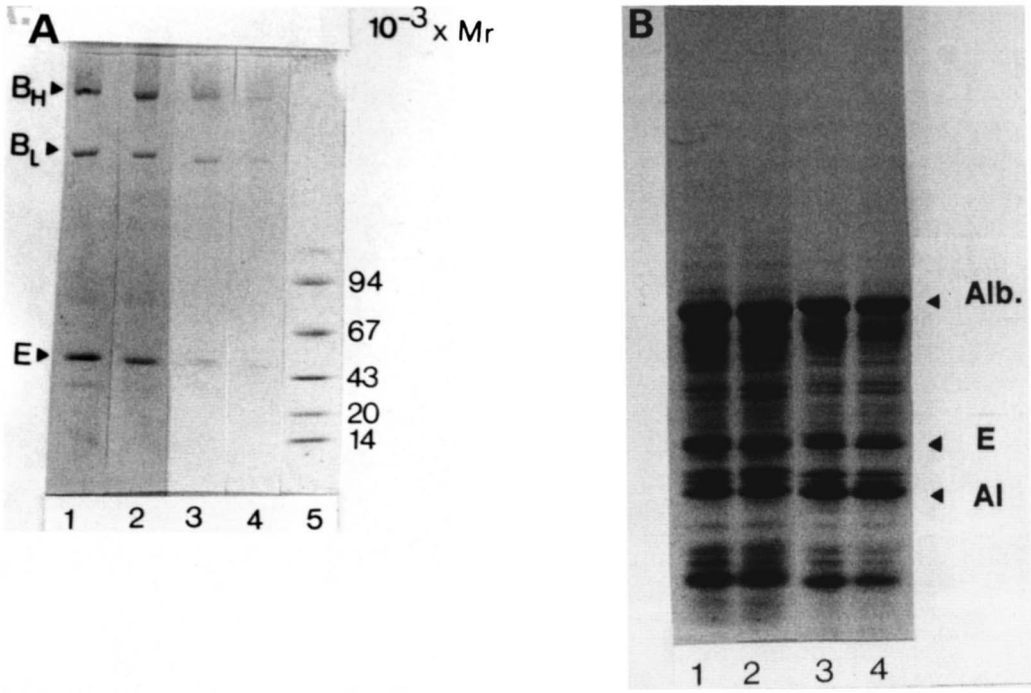

Fig. 4. Polyacrylamide gel electrophoresis of apoproteins secreted by hepatocytes. Lipoproteins secreted after 5 h were isolated from the culture medium of cp/cp and lean rat hepatocytes by ultracentrifugation. The lipoproteins were fractionated into density classes and concentrated by addition of Cab-O-Sil. Proteins were extracted from the Cab-O-Sil and subjected to electrophoresis on a 3–15% gradient polyacrylamide gel containing 0.1% sodium dodecyl sulfate. The apoproteins were visualized by Coomassie blue staining. Panel A is nascent VLDL; panel B is HDL. Lipoproteins from corpulent hepatocytes are in lanes 1 and 3 whereas lipoproteins from lean hepatocytes are in lanes 2 and 4. Samples from male rats are in lanes 1 and 2; samples from female rats are in lanes 3 and 4. Lane 5 of panel A shows protein standards with molecular weights indicated by the adjacent numbers. The data presented are from one of three similar experiments. The intensities of the bands corresponding to $apoB_H$, $apoB_L$, and apoE were measured at 550 nM and the areas were integrated. The areas under the peaks (10^{-3} × OD_{550} nM units) were as follows. For the male corpulent cells $apoB_H$ = 845; $apoB_L$ = 370; apoE = 811. For male lean cells $apoB_H$ = 584; $apoB_L$ = 63; apoE = 456. For female cp/cp cells $apoB_H$ = 185; $apoB_L$ = 53; apoE = 220.

to a general increase in secretion of all secretory proteins, since there was no obvious difference in the mass or [^{3}H]leucine incorporation into apoA-I secreted in the HDL of corpulent and lean hepatocytes (Figs. 4, 5C, 6C). Nor was there an increase in the amounts of total Cab-O-Sil-bound proteins in the culture medium of corpulent, compared to lean, cells (**Table 3**). In the albumin secreted into the fraction of density > 1.18 g/ml from the male cp/cp and control hepatocytes there were 5153 ± 2215 and 5626 ± 1518 dpm/mg cell protein, respectively. For cells from the female rats, the albumin from the cp/cp and control cells contained 3333 ± 520 and 2652 ± 902 dpm/mg cell protein, respectively.

The increased secretion of [^{3}H]leucine-labeled apoproteins into VLDL cannot be explained by a general increase in cellular protein synthesis in corpulent versus control cells. The data presented in Table 3 demonstrate that the incorporation of [^{3}H]leucine into cellular proteins of cp/cp hepatocytes, both male and female, was no higher than in lean cells. On the contrary, there was a slightly lower, but not statistically significant ($P > 0.05$), incorporation of [^{3}H]leucine into the cellular proteins of the cp/cp, compared to the control, hepatocytes.

DISCUSSION

Increased secretion of VLDL lipids and apoproteins from cp/cp hepatocytes

Isolated hepatocytes cultured from corpulent cp/cp male and female rats secrete abnormally large amounts of VLDL lipids and apoproteins compared to hepatocytes from lean, control rats. The rate of TG secretion from the male and female cp/cp rat hepatocytes was 3 to 4 times as high as from the lean cells, as measured by mass and [^{3}H]glycerol incorporation. In addition, the rate of PtdCho secretion from both male and female corpulent rat hepatocytes was higher than from lean cells. In intact animals the apparent hepatic TG and phospholipid secretion rates have been previously measured after Triton WR1339 inhibition of lipoprotein lipase (3). The differences in lipid secretion rates observed in both isolated hepatocytes and intact animals are in agreement with, and may account for, the pronounced hyperlipidemia observed in the blood of these animals (2). The reason for the significantly higher TG content in the female cp/cp cells versus either the female lean, or male corpulent, cells is not known.

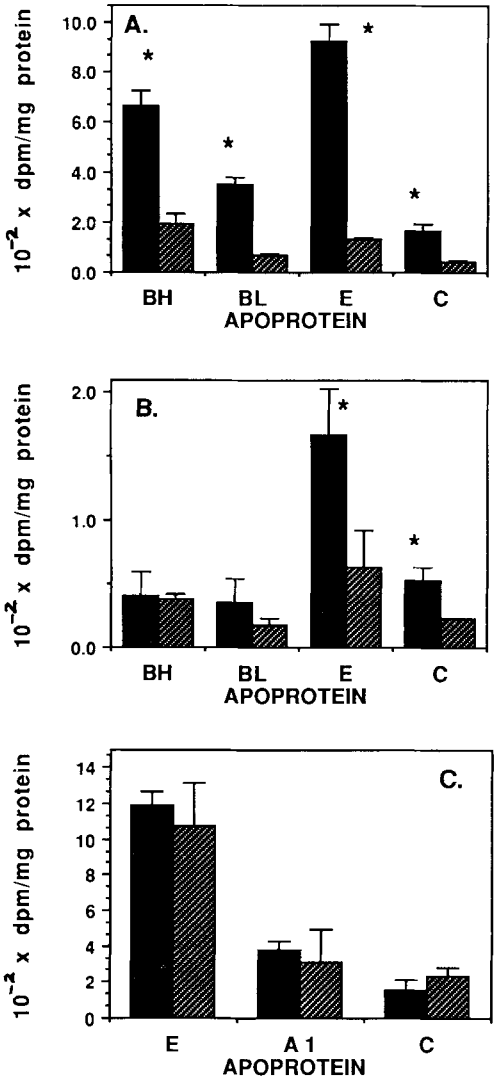

Fig. 5. Incorporation of [4,5-^{3}H]leucine into apoproteins of VLDL, LDL, and HDL fractions from culture medium of hepatocytes prepared from male corpulent (cp/cp) and lean (+/+) rats. Lipoprotein classes were separated on the basis of density from the culture medium of eight dishes of hepatocytes incubated for 5 h with [^{3}H]leucine (15 μCi/dish). Lipoproteins were concentrated by addition of Cab-O-Sil and apoproteins were solubilized from the Cab-O-Sil in a buffer containing 2% sodium dodecyl sulfate and 6 M urea. Standard lipoproteins, prepared from rat serum, were added as carriers and the samples were subjected to polyacrylamide gel electrophoresis on 3–15% gradient gels containing 0.1% sodium dodecyl sulfate. The gels were stained with Coomassie blue and the bands corresponding to $apoB_H$, $apoB_L$, apoE, apoC, and apoA-I were cut from the gels and counted for radioactivity. Panel A, VLDL; panel B, LDL; panel C, HDL. Statistically significant differences were determined by the unpaired Student's *t*-test. * Refers to $P \leq 0.0005$. The incorporation of [^{3}H]leucine into all other apoproteins was not significantly different between lean and corpulent cells.

The secretion of VLDL apoproteins ($apoB_H$, $apoB_L$, apoE, and apoC) was significantly increased in the corpulent, compared with the lean, hepatocytes. This difference cannot be explained by a general increase in cellular protein synthesis in the cp/cp, compared to the lean, cells as measured by the incorporation of [^{3}H]leucine into total trichloroacetic acid-precipitable cellular material. Nor was there a general increase in protein secretion by the cp/cp hepatocytes.

In contrast to the present study in which there was an increased secretion of apoB by the cp/cp hepatocytes, in the serum of the intact cp/cp rats the level of apoB was not significantly higher in the corpulent rats than in the lean rats (2). However, the amount of apoE in the cp/cp rat serum was markedly increased compared with that from the lean rats (2).

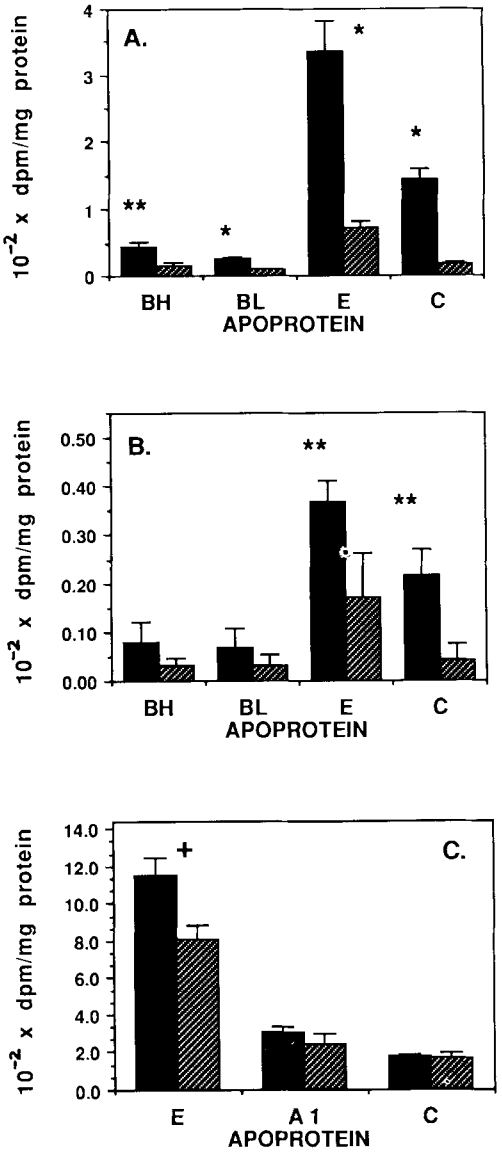

Fig. 6. Incorporation of [4,5-^{3}H]leucine into apoproteins of VLDL, LDL, and HDL fractions from the culture medium of hepatocytes prepared from female corpulent (cp/cp) and lean (+/?) rats. The experiment was performed exactly as described in the legend to Fig. 5, except that female rats were used. Statistically significant differences were determined by the unpaired Student's *t* test. * Denotes $P \leq 0.0005$; **, $0.0005 < P \leq 0.005$; *, $0.005 < P \leq 0.05$.

TABLE 3. Incorporation of [4,5-^{3}H]leucine into hepatocyte cellular and secreted proteins

Time of Incubation	Male Rats		Female Rats	
	cp/cp	+/+	cp/cp	+/?
h	*10^{-3} × dpm/mg cell protein*			
2 (cells)	175.0 ± 4.3	205.0 ± 27.6	186.3 ± 12.1	212.2 ± 46.5
6 (cells)	279.3 ± 39.0	328.1 ± 66.0	264.6 ± 27.4	330.7 ± 20.0
24 (cells)	451.3 ± 107.5	494.0 ± 10.1	491.8 ± 71.2	540.0 ± 97.5
6 (medium)	8.4 ± 0.8	8.0 ± 1.9	8.5 ± 1.5	11.8 ± 0.2

One dish of hepatocytes from each of corpulent and lean, male and female, rats was incubated with 5 μCi/dish of [4,5-^{3}H]leucine for the indicated times. Cells were washed and scraped from the dishes. Cellular protein was precipitated with 10% trichloroacetic acid, washed free of labeled leucine, and solubilized for radioactivity determination. The incorporation of ^{3}H into secreted proteins (6 h) was measured for medium lipoproteins obtained by addition of Cab-O-Sil to total culture medium. Values are averages ± SD from triplicate measurements of three independent experiments.

Throughout these studies the secretion of VLDL apoproteins by the male cells was consistently higher than by the female cells, whether measured as mass (Fig. 4) or [^{3}H]leucine incorporation. In contrast, the rate of secretion of TG by the female cells was higher than in males. Thus, the female VLDL particles are apparently TG-rich compared to VLDL secreted by male cells. This finding, in conjunction with the observation that approximately equal amounts of HDL apoproteins are present in the HDL fractions from male and female cells, may be significant in light of the increased susceptibility of the male rats to atherosclerosis.

Secretion of HDL components

Although the secretion of VLDL components was greatly increased in the corpulent, compared with the lean, hepatocytes, there was no significant difference in secretion of either lipid or apoprotein moieties of HDL. In contrast, the lipid levels in serum HDL of cp/cp rats were significantly higher than in lean rats. The explanation for these differences between the isolated hepatocytes and the intact rats in probably that there is a complex metabolism of lipoproteins in the circulation of intact animals that does not occur to the same extent with nascent particles secreted by isolated hepatocytes. The higher rate of VLDL secretion in the corpulent, compared to the lean, rats could result in production of more HDL particles, due to catabolism of VLDL in the serum. In addition, there may be different hormonal effects on HDL secretion and/or metabolism in the two types of animals, which would not be apparent in the isolated hepatocyte system. Moreover, the possibility must be considered that intestinal lipoprotein secretion might contribute to the hyperlipidemia observed in cp/cp rats. Indeed, the rate of intestinal lipoprotein secretion might also be increased in these animals.

The secretion of VLDL and HDL may be independently regulated. Other examples of differences between the secretion of hepatic VLDL and HDL have been previously reported. For example, in choline-deficient hepatocytes the secretion of all VLDL apoproteins and lipids was markedly reduced compared to that in choline-supplemented cells, whereas secretion of HDL apoproteins and lipids was unaffected by choline deficiency (17). Similarly, orotic acid treatment of rats reduced the secretion of VLDL, but not HDL, into liver perfusates (18). Moreover, we have recently demonstrated that dexamethasone treatment of rat hepatocytes increased the secretion of apoB and TG into VLDL by 4-fold and approximately 2-fold, respectively, but had a much smaller effect on the secretion of HDL components (P. Martin-Sanz, J. E. Vance, and D. N. Brindley, unpublished observations). Thus, evidence is accumulating that hepatic VLDL and HDL are probably assembled and secreted by different mechanisms.

Hyperlipidemia in intact animals is also evident in isolated hepatocytes

A significant finding is that the hyperlipoproteinemia observed in intact, corpulent rats persisted when isolated hepatocytes were cultured from the livers of these animals. In the intact cp/cp rat it is difficult to discern whether the apparent hypersecretion of TG (3) is due to an inherent defect in hepatic metabolism or whether the abnormality is the result of differences in hormonal or nutritional status of the animals. An advantage of using isolated hepatocytes from these animals is that studies can be performed under controlled conditions, independent of hormonal or substrate variations. The cells used in the present studies were plated for approximately 20 h before the experiments were begun, thus most residual variability in the hormonal status of the animal would have been eliminated. The difference in secretion of TG between the corpulent and lean hepatocytes was sustained even after the cells had been in culture for 48 h or 72 h before the experiments were initiated. Most likely, therefore, the in-

creased secretion of VLDL from the cp/cp hepatocytes is due to hormonal differences. As shown in Fig. 2, there was initially a markedly higher rate of incorporation of [^{3}H]glycerol into cellular TG in the cp/cp hepatocytes compared to the control cells, which may either reflect a higher rate of TG synthesis in the corpulent rats or a smaller pool size of a glycerol-derived precursor of TG.

Recently, lipoprotein secretion by cultured hepatocytes prepared from lean and obese (fa/fa) Zucker rats has been studied (19). In homozygous fa/fa rats there was increased storage of TG in the liver compared to the lean rats, and an increased accumulation of TG in the plasma (20). In hepatocytes cultured from these animals the secretion of TG was 2-fold higher in the obese, than in the lean, cells. The underlying biochemical defect in these rats is not yet known (19). However, in contrast to the present study, there was no increased secretion of VLDL apoproteins by the fa/fa hepatocytes (19). In addition, the Zucker obese rats do not spontaneously develop atherosclerotic or ischemic myocardial lesions (21).

In the present study with cp/cp rat hepatocytes (see Fig. 2A), and in the study with the fa/fa hepatocytes (19), the cp/cp and fa/fa cells initially incorporated more [^{3}H]glycerol into cellular TG than did the lean cells, suggesting differences in TG metabolism. Five hours after addition of [^{3}H]glycerol, there was a more rapid decline in the amount of [^{3}H]TG in the fa/fa cells than in the lean cells, and a concomitantly increased accumulation of labeled TG in the medium (19). An analogous result is shown in Figs. 2A and B of the present study. In the hepatocytes from each of the lean and corpulent male rats, the loss of glycerol-labeled TG from the cells could be almost quantitatively accounted for by secretion of labeled TG.

The specific radioactivity (dpm/nmol) of the cellular and secreted TG synthesized from [2-^{3}H]glycerol was calculated for the corpulent and lean male hepatocytes (**Fig. 7**). At all times, except after 24 h, the specific radioactivity of cellular TG was as high as, or higher than, that in culture medium. Thus, there was no evidence that newly synthesized TG was selected for secretion in preference to pre-existing TG; had this been the case, the secreted TG would have been of a higher specific radioactivity than cellular TG. Apparently, newly made and "old" TG have become rapidly mixed. This result is in contrast to the observation of Ide and Ontko (22) in perfused rat liver, in which a preference for secretion of newly synthesized, rather than pre-existing, TG was reported. Previously published work (17, 23–25), has suggested a linkage between new synthesis of phospholipids and their assembly and secretion into lipoproteins.

In conclusion, isolated hepatocytes prepared from the atherosclerosis-prone JCR:LA-corpulent (cp/cp) rats have a several-fold increased rate of secretion of apoprotein and lipid components of VLDL, compared to hepatocytes prepared from lean rats. No significant differences in secretion of HDL lipids or apoproteins were observed between the two types of cells. The differences in lipoprotein levels in the serum of the intact animals may therefore be the result of increased hepatic secretion of VLDL. The differences in VLDL secretion are most likely not the result of hormonal differences since these would not be expressed in hepatocytes cultured under the conditions used in the present experiments. Although the biochemical basis for the differences in VLDL secretion in the corpulent and lean rats is not yet understood, there appears to be an inherent difference in glycerolipid metabolism in the two types of hepatocytes. Future experiments on the metabolism of the glycerolipids in isolated hepatocytes from these animals should provide some information on what factors regulate VLDL triacylglycerol and apoprotein secretion and what is the basic biochemical defect underlying the hyperlipidemia in the cp/cp rats. From information presently available it appears that under most metabolic conditions apoB is synthesized constitutively (26), and in excess, so that the rate of VLDL secretion is probably not controlled by the rate of apoB synthesis, but rather by the availability of lipid.

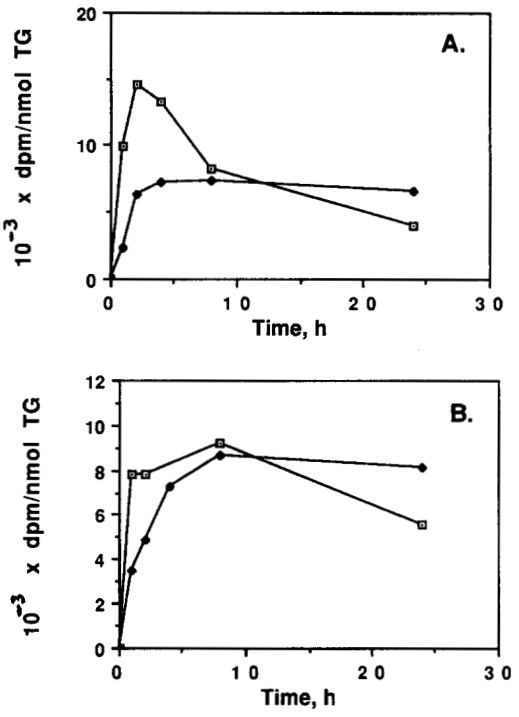

Fig. 7. Specific radioactivity of cellular and secreted TG from [2-^{3}H]glycerol-labeled hepatocytes from male cp/cp and +/+ rats. The specific radioactivity of TG in cells (panel A) and medium (panel B) was calculated from the radioactivity data in Fig. 2 and the mass data in Fig. 1A and Table 1. The following symbols are used: □ = cp/cp; ◆ = +/+.

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