Secretion of apolipoproteins in very low density and high density lipoproteins by perfused rat liver

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Abstract The incorporation of labeled amino acids into the peptides of very low density lipoproteins (VLDL) and high density lipoproteins (HDL) secreted by perfused rat liver was studied using a Ringer–albumin solution in the perfusate in place of serum to diminish exchange of peptides between VLDL and HDL. Among the lipoproteins, the greatest release of protein, greatest incorporation of amino acid, and highest specific activity were found in VLDL. After separation of the delipidated peptides by electrophoresis on polyacrylamide gel, the incorporation into VLDL peptides was found to be 5–10 times as great as into HDL peptides. There was virtually no incorporation into the peptides of low density lipoproteins (LDL). Approximately 25% of the radioactivity incorporated into perfusate VLDL failed to enter the 13% polyacrylamide gel. The remaining radioactivity was distributed primarily among three peptide bands; one, found in the upper portion of the gel, contained 45% of the total, most of the remainder being found in two rapidly migrating bands. These three peptides appear to approximate those of human apo-C in relative electrophoretic mobility. Most of the HDL peptide radioactivity entering the running gel was found in a band that migrates slightly faster than the main VLDL band. A portion of the radioactivity of this major HDL band did not enter the running gel unless β-mercaptoethanol was present. Greater separation of these two bands by polyacrylamide gel electrophoresis for 24 hr confirmed that the major bands in VLDL and in HDL were different. The rapidly moving peptides of HDL were found to contain very little radioactivity. Determination of the intensity of staining of carrier-free perfusate VLDL and HDL peptides produced a pattern similar to the incorporation of labeled amino acids. It is concluded that the rapidly moving peptides, which may contain activators of lipoprotein lipase, are only secreted as part of the VLDL.

Supplementary key words gel electrophoresis

It is now well established that the liver is the main site of synthesis of very low density lipoprotein (1, 2). The appearance of VLDL-like particles in the Golgi apparatus of perfused liver and their subsequent release into perfusates have been demonstrated by Jones, Ruderman, and Herrera (3). On the other hand, LDL and HDL found in the liver perfusate or circulation theoretically may originate from two sources: direct secretion or the catabolism of VLDL by the action of lipoprotein lipase (4). Windmueller, Herbert, and Levy (5) have recently reported approximately equal incorporation of radioactive lysine into the VLDL and HDL of liver perfusates. Haft et al. (6), while finding the greatest incorporation of lysine into the VLDL of rat liver perfusates, also found considerable radioactivity in the LDL and HDL fractions. However, lipase may be released from the liver (7, 8) by the use of heparinized blood in the perfusates. Wilcox, Fried, and Heimberg, (9) and Kook and Rubinstein (10), using defibrinated blood and an erythrocyte–albumin–Krebs-Ringer solution, respectively, noted considerably less incorporation of radioactive amino acids into the perfusate HDL than into VLDL, although, in the rat, circulating HDL contains most of the protein moiety of the total lipoproteins (11, 12). Evidence supporting the suggestion that HDL is directly secreted by the liver is provided by the observation that administration of orotic acid, which inhibits secretion of β-lipoprotein, apparently does not prevent the appearance of α-lipoproteins in the perfusate (13).

The protein moieties of VLDL and HDL, both in the human (14, 15) and in the rat (16, 17), are made up of at least six peptides, each of which can readily be separated, after delipidation of the lipoproteins, by electrophoresis on polyacrylamide gel (18, 19). Thus, it has been shown that VLDL isolated from the Golgi apparatus of perfused livers has the same pattern of peptides as that found in the plasma (20). The ability of perfused liver to incorporate precursor amino acids into the peptides of perfusate VLDL and HDL has recently been demonstrated (5, 21). However, analysis of the patterns of incorporation of the amino acids into the various peptides of secreted VLDL and HDL by perfused rat liver is complicated when the perfusate contains serum, since it has been shown that there is an exchange between VLDL and HDL of three of

Abbreviations: VLDL, very low density lipoproteins (d < 1.006); LDL, low density lipoproteins (d 1.006–1.09); HDL, high density lipoproteins (d 1.09–1.21); SDS, sodium dodecyl sulfate; β-ME, β-mercaptoethanol.

the peptides separable by polyacrylamide gel electrophoresis (18).

The degree to which the various peptides of the circulating lipoproteins are synthesized and secreted by the perfused rat liver was therefore compared. A perfusate made up of Krebs-Ringer bicarbonate solution containing bovine serum albumin and washed rat erythrocytes was used to minimize the difficulties inherent in the use of either serum or heparinized plasma. Under these conditions it was found that the patterns of secretion of the peptides of two classes of lipoproteins differed in several significant respects. These data will be presented in this communication. Preliminary reports have been presented (22, 23).

METHODS

Male hooded rats fed ad lib. were used as donors of livers and erythrocytes. The livers were perfused for 3 hr by the method of Miller et al. (24), using 144 ml of perfusate containing 20% washed rat erythrocytes, 3% albumin, 0.5% glucose, and 80 mg of a mixture of essential amino acids[1] (25) dissolved in Krebs-Ringer bicarbonate buffer. The gas phase was 95% O_2–5% CO_2. No heparin was used in vivo or at any time during the collection of blood, perfusion, or isolation of lipoproteins. Perfusate lipoprotein, as well as unlabeled rat serum lipoproteins used as carriers, was isolated by the method of Havel, Eder, and Bragdon (26), using an IEC B-60 preparative ultracentrifuge with an SB-283 rotor at 15°C and 33,000 rpm. VLDL was isolated by centrifugation for 18 hr at a density of 1.006. The density of the infranatant solution was then raised to 1.09, and the solution was centrifuged for 24 hr. Radioactivity in the d 1.006–1.09 fraction, containing the LDL and a trace of HDL, was found to be slight (approximately 3% of the total lipoprotein radioactivity) and was discarded. A density of 1.09 was selected for isolation of HDL to minimize contamination by LDL (27). The remaining solution was then adjusted to a density of 1.21 and centrifuged for 48 hr to collect the HDL. The isolated VLDL and HDL were washed by recentrifugation at densities of 1.006 and 1.21, respectively. Isolated serum VLDL or HDL was added as carrier prior to either the washing or the delipidation of the corresponding serum lipoproteins. The concentration of the carrier was adjusted so that 200–300 μg of delipidated peptides was applied to each polyacrylamide gel column, with a significant number of cpm in each resulting band. The resulting isolated lipoproteins appeared to be free from contamination by nonlipoprotein material when tested by immunodiffusion against rabbit antisera to rat serum and rat albumin. Delipidation of the lipoproteins, electrophoresis of the apoproteins in urea, and determination of the radioactivity in the polyacrylamide gel bands were carried out by the procedures previously described (18, 21), except that 1% β-ME was present in the 7 M urea solution used to dissolve the delipidated HDL, and 0.1% β-ME was added to the buffer in the upper compartment of the electrophoresis apparatus. Less than 10% of the radioactivity was lost during delipidation. The recovery of radioactivity applied to the gels was 85–110% (avg 94%) for VLDL and 80–105% (avg 91%) for HDL. These recoveries include the radioactivity found in the stacking gel and at the top of the running gel. In some experiments the delipidated peptides were dissolved by heating for 10 min at 100°C in a 0.1 M sodium phosphate buffer containing 1% SDS and 1% β-ME. The peptides were then separated by electrophoresis on 10% polyacrylamide gels containing SDS and prepared according to the method of Dunker and Rueckert (28), using the buffer system of Fairbanks, Steck, and Wallach (29). The intensity of the staining of the peptides on the gels was measured using a Beckman Acta III densitometer. Proteins were determined by the method of Lowry et al. (30), using 2% deoxycholate to clear the lipids in VLDL.

RESULTS

When this work was first undertaken, it was felt that the use of a perfusate in which serum was replaced by a Ringer solution would minimize the exchange of peptides between VLDL and HDL as well as the action of lipoprotein lipase, possibly altering the distribution of labeled amino acid precursors among the lipoproteins from that

TABLE 1. Incorporation of labeled amino acids into lipoprotein peptides secreted in vivo and by perfused liver

	% of Radioactivity	
	Perfused Liver	In Vivo
VLDL	83 ± 2.5	43 ± 2.7
LDL	2 ± 0.3	12 ± 2.7
HDL	15 ± 2.5	45 ± 2.9
Number of experiments	4	5

For the in vivo experiments, 25 μCi of [1-^{14}C]leucine or 50 μCi of [^{3}H]valine was injected intravenously 90 min before the rat was bled. The perfusate contained 100 μCi of [3,4-^{3}H]leucine or 50 μCi of [1-^{14}C]valine in 72 ml of perfusate. Perfusion time, 3 hr. The lipoproteins were delipidated before the radioactivity in the peptides was assayed. In some experiments, ^{3}H- and ^{14}C-labeled amino acids were used in the same animal or perfusate. No significant differences were noted between the two labels. Results are means ± SEM.

[1] Free fatty acids were not added to the perfusate used in the experiments to be reported. However, a series of perfusions was carried out in which albumin-bound fatty acids were added to the perfusate as a bolus at the start and then as a continuous infusion throughout the perfusion. The pattern of incorporation of amino acids into the various peptides of VLDL was not significantly affected, although the amount of VLDL collected from the perfusate was markedly increased.

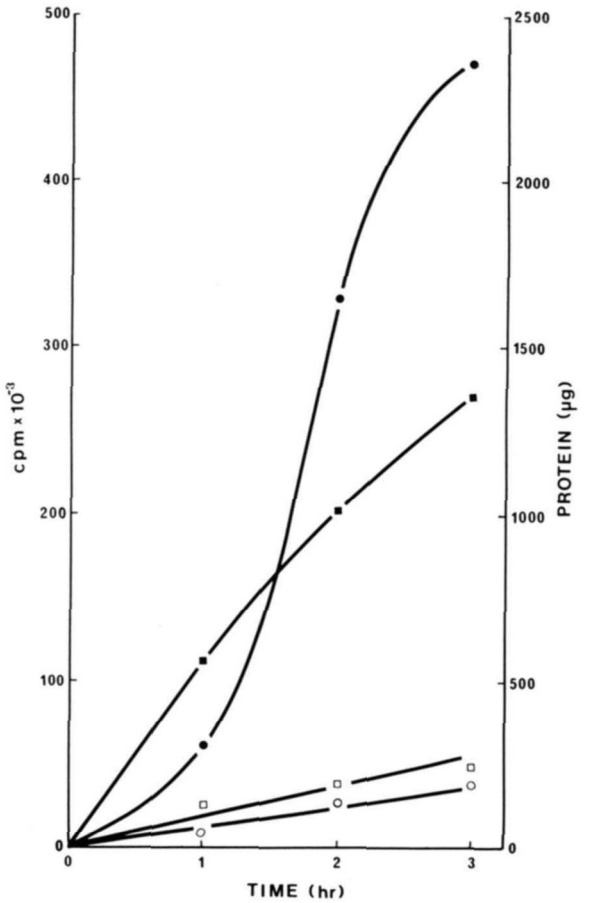

Fig. 1. Protein and radioactivity of VLDL and HDL released during a 3-hr perfusion in the presence of [1-^{14}C]leucine. ●, VLDL radioactivity; ■, VLDL protein; ○, HDL radioactivity; □, HDL protein. The experiment is typical of three such perfusions. 100 μCi of [1-^{14}C]leucine was dissolved in 144 ml of perfusate.

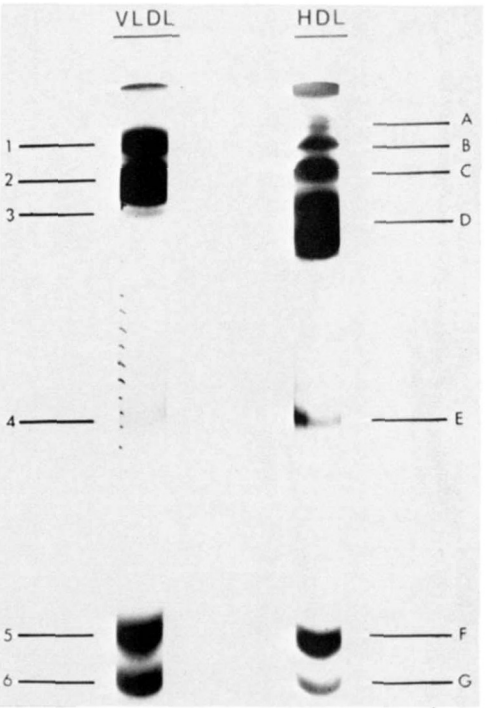

Fig. 2. *Polyacrylamide gel electrophoresis patterns of delipidated rat serum VLDL and HDL dissolved in 7 M urea. The system of designating VLDL bands by numbers and the HDL bands by letters is a tentative one used for convenience in describing the work presented here.*

seen in vivo. Thus, the incorporations of [1-^{14}C]leucine into the lipoprotein fractions in vivo and in vitro were compared. The results are shown in **Table 1.** It will be noted that after 3 hr of perfusion only 3% of the isotope among the d < 1.21 lipoproteins was found in the LDL, 13% in the HDL, and the remaining 85% in the VLDL. In contrast, in vivo, although the percentage of lipoprotein radioactivity in LDL was considerably higher, most of the incorporation was equally distributed between HDL and VLDL. Since the in vivo distribution resembles that reported by Windmueller et al. (5) for livers perfused with serum, it appears that exchange is diminished by the absence of serum from the perfusate.

The incorporation of [^{14}C]leucine and release of VLDL and HDL proteins during the course of a 3-hr perfusion are shown in **Fig. 1.** In these experiments, isolated carrier lipoproteins were not used. It will be noted that there is a greater release of VLDL than HDL protein. The former also contains more radioactivity. Although the release of the VLDL and HDL protein was linear over the 3-hr period, the incorporation of [^{14}C]leucine into VLDL lagged behind the release of the peptide during the first hour. A delay in incorporation of [^{14}C]leucine into β-lipoproteins secreted in vivo as well as by perfused liver has previously been reported (31). After 3 hr of perfusion the rate of incorporation of [^{14}C]leucine into VLDL had increased sharply, so that the specific activity of the latter exceeded that of HDL. In three experiments of this type, the specific activity (cpm/μg of protein) of the VLDL after 3 hr of perfusion was 360 ± 17, while that of HDL was 160 ± 3.

The pattern of incorporation of [4,5-^{3}H]leucine into the various peptides of the perfusate lipoproteins was determined by delipidation of isolated VLDL and HDL, followed by their dissolution in urea and separation on polyacrylamide gel. **Fig. 2** shows a typical polyacrylamide gel pattern from rat lipoproteins. The VLDL bands are designated by numbers and the HDL bands by letters. This convention has been adopted in our laboratory pending the purification of the peptides and identification on a chemical basis such as the C-terminal amino acids, as has been done for many of the peptides for human apolipoproteins (32). It should be noted that, using this nomenclature, bands 1–6 of VLDL correspond in electrophoretic mobility to bands B–G, respectively, of HDL.

The patterns of incorporation of [^{3}H]leucine during the course of the perfusion into the various lipoprotein pep-

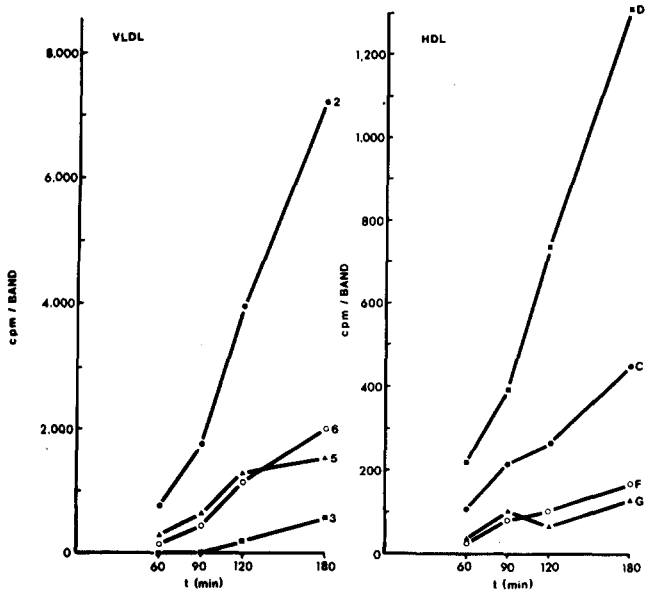

Fig. 3. Incorporation of [^{3}H]leucine into the peptides of perfusate VLDL and HDL. The experiment illustrated in the figure is typical of four such perfusions. Note the difference in scales on the ordinates. The values on the ordinates represent the cpm of each band in 40% of the perfusate. The cpm in the samples assayed represented 15% and 20% of the total cpm for VLDL and HDL, respectively. All samples were counted to a standard error of <1%. The perfusate contained 250 μCi of [3,4-^{3}H]leucine. Bands 1 and 4 of VLDL and A, B, and E of HDL are not shown because they contained little radioactivity.

tides that enter the running gel are shown in **Fig. 3.** It will be noted that in perfusate VLDL the greatest incorporation of [^{3}H]leucine was into the peptides of band 2, with significant additional radioactivity found only in the rapidly migrating bands 5 and 6. In contrast, the leucine was most rapidly incorporated into band D of HDL, which corresponds in mobility to band 3 of VLDL. The latter, however, has relatively little radioactivity and is measurable only after 120 min of perfusion. It should be noted that the overall incorporation in the VLDL was about six times as great as that found in HDL. This is reflected by the difference in units on the ordinates of Fig. 3.

Although the experiment cited in Fig. 3 utilized [^{3}H]leucine as the labeled precursor for peptide synthesis, the possibility that another amino acid might produce different results was considered. Perfusions were carried out in which the liver was simultaneously perfused with [1-^{14}C]valine and [4,5-^{3}H]leucine. No significant difference in the distribution of the two isotopes was noted. The pattern was also unchanged when a mixture of ^{14}C-labeled amino acids was used. The combined results of these experiments, expressed as percentages of the total incorporation into each lipoprotein class, are shown in **Table 2.** It will be noted that there is a consistent pattern of distribution of radioactivity among the peptides of VLDL, similar to that developed during the perfusion shown in Fig. 3, even when the material remaining at the top of the running gel, presumably apo-LDL, is taken into consideration. The apo-HDL radioactivity also resembles that shown in the time curve in Fig. 2, with 35% of the total incorporation in band D compared with only 4% in the corresponding band 3 of VLDL.

In a preliminary communication (23) we reported that in some experiments as much as 70% of the radioactivity of the secreted HDL peptides would not enter the running gel, with a concomitant loss of radioactivity primarily from band D. We have since noted that the material lost from band D can be restored if the apo-HDL is dissolved in urea containing 1% β-ME and then electrophoresed in the presence of 0.1% β-ME. **Table 3** shows the results of such a perfusion in which the delipidated HDL was dissolved and the peptides were separated by electrophoresis in the presence and absence of β-ME. Therefore, β-ME was added to 7 M urea used to dissolve the apoproteins in the experiments reported in this communication. However, β-ME failed to reduce the level of nonpenetrating labeled peptides below 30%.

The appearance of considerable radioactivity in the perfusate apo-HDL that does not enter the running gel was unexpected because there had been no report of a similar phenomenon in HDL labeled in vivo (18, 20). To deter-

TABLE 2. Distribution of labeled amino acids among peptides of perfusate lipoproteins

VLDL		HDL	
Peptide Band	% Incorporation	Peptide Band	% Incorporation
O[a]	23 ± 2.9	O	35 ± 2.1
		A	3 ± 0.4
1	2 ± 0.3	B	3 ± 0.2
2	45 ± 6.4	C	10 ± 0.8
3	4 ± 0.9	D	35 ± 2.2
4	1 ± 0.2	E	3 ± 0.6
5	12 ± 1.2	F	4 ± 1.2
6	15 ± 1.7	G	5 ± 1.3
Number of perfusions	8		6

Results are means ± SEM.
[a] O, radioactivity remaining at the top of the running gel or in the stacking gel.

TABLE 3. Comparison of distribution of [^{3}H]leucine among peptides of delipidated HDL dissolved in urea in presence and absence of β-ME

Peptide Band	% of Radioactivity	
	Without β-ME	With β-ME
O	63	40
A + B	5	5
C	9	9
D	17	39
E	1	2
F + G	5	4

mine if the presence of the high molecular weight material is specific to the perfused liver and not an artifact due to delipidation or other steps in the separation and analysis of HDL, the distribution of radioactivity among the HDL peptides after the simultaneous intravenous injection of [4,5-^{3}H]leucine and [1-^{14}C]valine in the rat was studied and is shown in **Table 4.** The identical techniques were used to isolate, delipidate, and separate HDL peptides as had been used in vitro. It will be noted that, after in vivo labeling, less than 10% of the radioactivity failed to enter the running gel. The major incorporation was again found in band D. These data correspond very closely to those obtained by Roheim et al. (19). The small amount (5–6%) of radioactivity found at the top of the running gel may correspond to that found by the latter in their band 1, because the present experiments utilized a 13% gel compared with the 10% gel used by Roheim et al. (19).

In an attempt to identify the HDL peptides remaining at the top of the running gel, aliquots of isolated HDL labeled in vivo with [1-^{14}C]leucine were reisolated before and after circulation in the perfusion apparatus for 3 hr in the absence of a liver. It was found that the repeated long ultracentrifugation of such small quantities of the HDL, whether or not the HDL had been suspended in the perfusate and with or without circulation in the perfusion apparatus, resulted in an increase of radioactivity retained at the top of the gel from 10% to 30–35%. The extra material originated from bands C and D in about equal quantities. It is of interest that if the percentages of radioactivity found in bands C and D of HDL secreted in vitro (see Table 2) are increased by about 10% to account for the material not entering the gels, the resulting values are very similar to those seen in HDL labeled in vivo (see Table 4). The quantity of apo-HDL radioactivity remaining at the top of the running gel was not reduced by dissolution and electrophoresis of the peptides in SDS unless diluted serum was used in the perfusate.[2] Our data differ from those of Windmueller et al. (5), who observed a considerably smaller proportion of apo-HDL radioactivity remaining at the origin of a 10% polyacrylamide gel, without the use of SDS.

The gels illustrated in Fig. 2 and used to derive the data presented thus far utilized isolated serum lipoproteins as carriers during the washing of the lipoprotein fraction and subsequent electrophoresis. In several experiments the lipoproteins were isolated from the entire perfusate and delipidated and the peptides were separated by electrophoresis without added carrier. The intensity of staining of the gels was determined. While these scans cannot be used to estimate accurately the amount of protein in each peptide band, they do provide some indication as to whether the quantity of protein correlates with the radioactivity

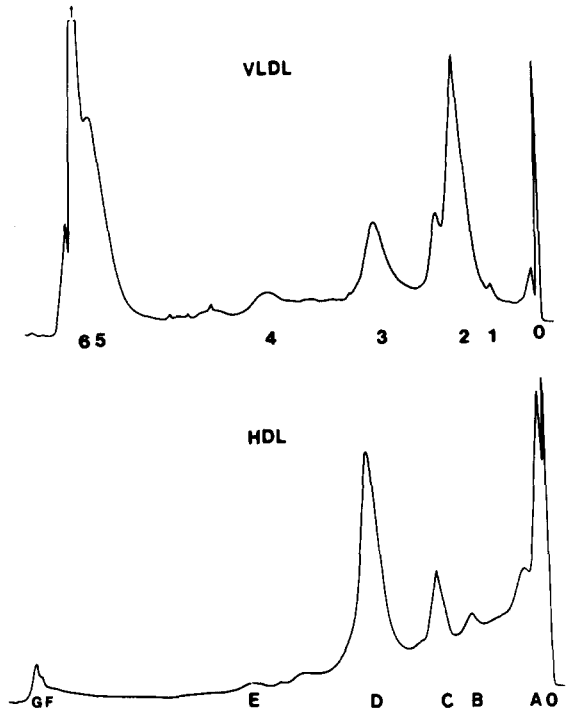

Fig. 4. Densitometer tracings of amido black-stained polyacrylamide gels of perfusate VLDL and HDL peptides obtained after 3 hr of perfusion.

recovered in the peptides. The scans of the gels of the perfusate VLDL and HDL peptides are shown in **Fig. 4.** It will be noted that bands 5 and 6 of VLDL make up an important portion of the total peptides of the VLDL, but the corresponding bands F and G in HDL are found only in very small quantities. In addition, the gels indicate that the main secreted peptides that enter the running gel are 2, 5, and 6 of VLDL and primarily D of HDL. Thus, the intensity of protein staining generally corresponds to the distribution of the labeled amino acids shown in Table 2.

Because of the relative proximity of bands 2 and 3 of VLDL and C and D of HDL, an attempt was made to

TABLE 4. Incorporation of [^{14}C]leucine and [^{3}H]valine into HDL peptides in vivo

Peptide Band	% of Radioactivity	
	Valine	Leucine
O	6	5
A	2	2
B	2	2
C	20	17
D	47	44
E	4	7
F	5	8
G	9	12
Total cpm in fraction	3300	13,100

Each animal received intravenously 25 μCi of [1-^{14}C]leucine and 50 μCi of [^{3}H]valine.

[2] Noel, S. P. Unpublished results.

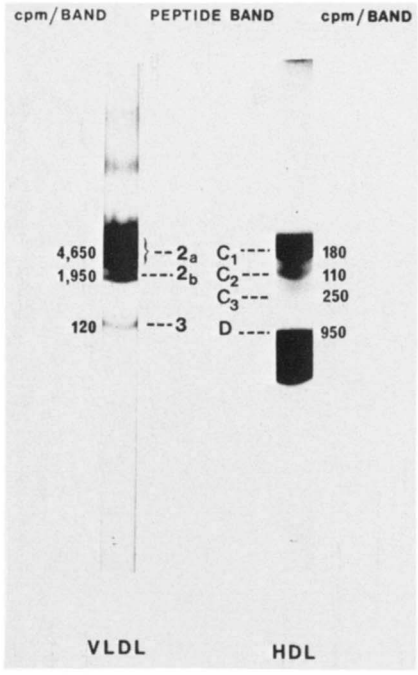

Fig. 5. Incorporation of [1-^{14}C]valine into peptides of bands 2 and 3 of perfusate VLDL and C and D of perfusate HDL after 24 hr of electrophoresis on 13% polyacrylamide gels. The radioactivity represents the total for each band of VLDL or HDL present after 3 hr of perfusion.

establish that the highly labeled bands in VLDL and HDL represent different peptides. The bands were further separated by allowing them to migrate for 24 hr during the electrophoresis on polyacrylamide gel. The resulting patterns and distribution of radioactivity are shown in **Fig. 5.** The prolonged electrophoresis results in one minor band, designated 2_b, splitting off from band 2 of VLDL, while band C of HDL is split into three, designated C_1, C_2, and C_3. These are separate from bands 3 and D of VLDL and HDL, respectively. It will be noted that radioactivity of VLDL is found primarily in band 2_a, with a lesser amount in 2_b. VLDL does not have a peptide band corresponding in mobility to band C_3 of HDL. Nevertheless, this area of the gel was routinely counted but contained little radioactivity. HDL bands C_1–C_3 contain relatively little radioactivity when compared with either the corresponding VLDL bands or HDL band D.[3] Thus, the difference in incorporation of amino acids into the major peptides of VLDL and HDL in the upper part of the gels is maintained when these bands are more widely spaced.

DISCUSSION

The present report confirms earlier ones (5, 6, 9, 10, 21) that the liver secretes HDL, but the rate of incorporation of the precursor amino acids into the HDL peptides is about one-tenth to one-fifth that of the VLDL peptides. This contrasts somewhat with data recently reported by Windmueller et al. (5), who found an equal incorporation of [^{14}C]lysine into the d < 1.006 and d 1.06–1.21 fractions of the perfusate lipoproteins. This difference is probably due to the utilization by Windmueller et al. (5) of a perfusate containing heparinized plasma or defibrinated serum. Serum or plasma contains a large reservoir of unlabeled peptides, some of which can undergo exchange with the secreted labeled peptides of the VLDL (18). This could also account for the observation by Windmueller et al. (5) that the peptides of group III of HDL (bands F and G in the present nomenclature) contain a higher percentage of radioactivity than do the corresponding peptides of VLDL. These are among the peptides that undergo exchange in rat (18, 19) and in human (33). The administration of labeled leucine in vivo results in a distribution of the radioactivity among VLDL, LDL, and HDL corresponding to that obtained by Windmueller et al. (5) in perfusates containing serum or plasma.

There are several significant differences between the patterns of incorporation of the amino acids into the peptides of VLDL and HDL. Among the peptides entering the running gel, band 2 of VLDL has the greatest incorporation, while band D, which differs slightly in electrophoretic mobility, has the greatest activity in HDL. Even though both appear to have approximately the same molecular size (17), the 24-hr gel electrophoresis, which results in a better separation of peptides, confirms that the VLDL and HDL bands having the highest radioactivity are different. The time curves of secretion of these peptides also differ (see Fig. 2). The secretion of an apoprotein migrating as band D distinguishes HDL from VLDL. It is of interest that band D resembles in electrophoretic mobility peptide P IV of Koga, Bolis, and Scanu (34), which cross-reacts with antiserum to apo-HDL but not with the antiserum to apo-VLDL. The pattern of incorporation of amino acids among the peptides of liver HDL is similar to that reported by Windmueller et al. (5) for the gut lymph HDL; both contain an insignificant amount of the small rapidly migrating peptides.

Based on the incorporation of labeled amino acids, it would appear that the main peptides of rat VLDL are those remaining at the origin, corresponding to human apo-B, and bands 2, 5, and 6, possibly corresponding to human apo-C (35). Furthermore, densitometric analysis of the stained polyacrylamide gels of perfusate HDL and VLDL peptides indicates that the fast-moving peptides represent a significant proportion of the total quantity of peptides in VLDL but a negligible amount in HDL. Herbert et al. (36) recently reported that C apolipoproteins isolated from rat serum HDL contained three major electrophoretic bands. A band designated C-I migrated more

[3] In some perfusions where incorporation was found in band C of HDL (21), it was primarily in C_3, which is not present in VLDL.

slowly than A-II of HDL;[4] apolipoproteins C-I and A-II possibly correspond to the HDL bands labeled C and D, respectively, in the present report. The other two bands migrated more rapidly; one contained peptides labeled C-II and C-III$_1$, and the other C-III$_2$. C-II activated lipoprotein lipase (36). Although these peptides have been isolated from rat HDL, the perfused liver results suggest that they were initially secreted primarily as VLDL. The peptide content of the electrophoretic band containing C-II is increased when VLDL is isolated from the serum of sucrose-fed rats (37).

The appearance of the rapidly migrating small peptides in the VLDL is consistent with the observation that VLDL peptides taken from the Golgi apparatus of hepatocytes have the same complement of peptides as circulating VLDL (20). It is not possible to determine from the present data whether the secreted VLDL would show an additional uptake of small peptides if exposed to serum, as has been suggested for nascent liver VLDL by Hamilton (38). However, the distribution of rapidly migrating peptides between VLDL and HDL is consistent with the concept advanced by Havel (39) and Havel, Kane, and Kashyap (40) that peptides that activate lipoprotein lipase originate from VLDL and undergo recycling through HDL, presumably after the action of the enzyme.

In some perfusions, much of the radioactivity of band D of apo-HDL was found in the peptide that does not penetrate the running gel (21) unless β-ME is used. This suggests that these peptides have sulfhydryl groups that may form extensive disulfide linkages. The presence of disulfide bonds in human apo-HDL has been demonstrated by Scanu, Lim, and Edelstein (41), although the aggregates of rat apo-HDL, appearing in the void volume after filtration on Sephadex G-200,[2] are much larger than the molecular weight of 17,000 reported by Scanu et al. (41) for the human apo-HDL peptide. It is not possible to determine from the present data whether some of the peptides of band D are secreted initially as polymers containing disulfide cross-linkages, then reduced in the circulation, whether a mixture of monomers and polymers are secreted, or whether the oxidation of sulfhydryl groups occurs during the perfusion of ultracentrifugation. A small amount of aggregated material may also be present in the rat circulation, because Bersot et al. (17) obtained a peptide fraction from HDL (HS-1) that did not penetrate the gels and gave an immunological cross-reaction with another penetrating band but not with any of the smaller rapidly migrating peptides of HDL or VLDL.

The data presented here suggest that perfused rat liver secretes VLDL composed of a large peptide that does not penetrate the gel, presumably the equivalent of human apo-B, and three peptides that probably are the equivalent of human apo-C peptides: apo-LP-Ser, apo-LP-Glu, and apo-LP-Ala. On the other hand, the HDL secreted by the perfused liver in the absence of serum contains primarily one peptide having different electrophoretic mobility from the major VLDL peptides, with virtually no secretion of the fast-moving peptides.

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