In order to determine if plasma apolipoproteins could be synthesized by tissues other than liver and intestine, two known sites of lipoprotein biogenesis, $[^{1}H]$leucine incorporation into lipoproteins was measured in rats in which the small intestine was resected and the liver was removed from the circulation. No evidence for additional apolipoprotein sources was found. Two methods were then developed to determine the relative contributions of liver and intestine to individual apolipoproteins found in plasma. In one method, plasma proteins from liver and intestine were differentially labeled by the simultaneous administration of $[^{1}H]$leucine intraduodenally and $[^{14}C]$leucine intraportally. The relative organ contributions were calculated from the $^{1}H/$$^{14}C$ ratios measured in individual plasma apolipoproteins and in several reference proteins, plasma albumin and selected apolipoproteins from mesenteric lymph. In the second method, the patterns of apolipoprotein synthesis were determined in (a) isolated, perfused livers, (b) isolated intestinal segments in vivo, and (c) intact rats, respectively, from the distribution of incorporated $[^{1}H]$leucine in each preparation. The relative organ contributions required to account for the pattern observed in the intact animal were then calculated by multiple regression analysis. Results from the two complementary methods, both applied in fat-fed animals, were in good agreement.

The quantitative order of apolipoprotein synthesis in intact rats was apolipoprotein E (apo-E) $>$ apo-B $>$ apo-A-I $>$ apo-C $>$ apo-A-IV. Nineteen per cent of the total apolipoprotein pool was synthesized by intestine and 81% by liver. The per cent intestinal contribution to individual proteins was as follows:apo-A-IV, 98; apo-A-I, 56; apo-B, 18; apo-C-II, 10; apo-C-III-0, 7; apo-C-III-2$^{-}$, <10; apo-C-III-3, <1; and apo-E, <1. Apo-C-III analyses were uncertain, but little or none was derived from intestine. Since apo-A-I and apo-A-IV are major apolipoproteins, designated in accordance with the A, B, C, system suggested by Alaupovic (7), are apo-A-I,$^1$ apo-A-IV, apo-B, a group of low molecular weight apo-C peptides, and apo-E (the arginine-rich protein) (3, 5, 6).

The only organs so far identified that synthesize the apolipoproteins are liver and small intestine. Available data indicate that, in the rat, liver is capable of synthesizing all of the major apolipoproteins (8–10). The small intestine, studied during fat absorption, can also synthesize most of these proteins with the possible exception of apo-E and several of the apo-C peptides (6, 11, 12). The relative contribution by liver and intestine to each of the apolipoproteins remains uncertain, as does the possibility of contributions from other tissues.

The present studies in rats had two main objectives: (a) to determine if any tissues besides liver and intestine synthesized plasma apolipoproteins, using animals in which the intestine was resected and the liver was removed from the circulation (hereafter referred to as eviscerated, functionally hepatectomized rats); no evidence for additional sites of synthesis was found; (b) to determine the relative hepatic and intestinal contributions to each of the major apolipoproteins in the circulating pool. Two methods were developed for this latter purpose based on the following principles.

In Method I, plasma apolipoproteins of intestinal and hepatic origin were differentially labeled in vivo by simultaneously administering $[^{11}H]$leucine intraduodenally and $[^{14}C]$leucine intraportally. Thus, intestinally made proteins were preferentially labeled with $^{11}H$ and hepatically made proteins with $^{14}C$. The relative organ contribution to each plasma apoprotein was estimated from its $^{11}H$/$^{14}C$ ratio. For reference purposes, the ratio found in plasma albumin characterized a protein made in the liver and the ratio found in selected mesenteric lymph apolipoproteins characterized a protein made by the small intestine.

In Method II, the patterns of apolipoprotein synthesis, defined as the relative amounts of labeled leucine incorporated into the individual proteins, were determined in isolated rat liver, in an isolated jejunal segment preparation, and in intact rats. Correlating the observed values by multiple regression analysis, the relative organ contributions needed to account for the pattern observed in the intact animal were then calculated.

**EXPERIMENTAL PROCEDURES**

**Rats and Diet**

All rats were 300- to 380-g Osborne-Mendel males fed NIH-07 open formula stock diet until the day of surgery. The subsequent experimental protocol is described below. Light ether anesthesia was used for all surgical procedures unless otherwise indicated.

$^1$The abbreviations used are: apo, apolipoprotein; VLDL, very low density lipoproteins; HDL, high density lipoproteins.
The right jugular vein was cannulated with silicone rubber tubing (0.94 mm outer diameter), which had been tunnelled subcutaneously through a small skin incision at the midscapular area. Through an abdominal incision, ligatures were placed around the descending colon, superior mesenteric artery, coeliac artery, hepatic artery, portal vein, and the duodenum about 3 mm distal to the pylorus. The entire incision between the duodenal and colonic ligatures and its supporting mesentry were resected, the abdominal opening was sutured, and the rats were placed in restraining cages. A solution containing 0.9% NaCl and 2.8% glucose was infused continuously into the jugular vein (0.94 mm outer diameter), which had been tunnelled subcutaneously through a small skin incision at the midscapular area. Through an 0.94 mm outer diameter) attached to silicone rubber tubing (0.64 mm outer diameter) was inserted through a small incision in an ileal vein and advanced until the tip lay in the superior mesenteric vein. The duodenum and, in some animals, also the mesenteric lymph duct were cannulated with polyvinyl tubing (0.97 mm outer diameter) (15), with care being taken in the latter case to disrupt all observed accessory mesenteric lymph channels. All tubing exited the abdominal cavity through small stab wounds. The animals were allowed to recover for 16 to 20 h in restraining cages with continuous infusions of 10% glucose, 0.85% NaCl, 0.04% KCl (glucose/saline) into the duodenum at 2.3 ml/h, and 0.9% NaCl into the portal cannula at 0.4 ml/h. Then the duodenal glucose/saline infusion was replaced with a lipid emulsion (Intralipid) providing 450 μmol of fatty acid/h. Two hours after the start of the lipid infusion, 1 mCi of L-[1,4,5-3H]leucine (120 Ci/mmol) in 1 ml of Intralipid was administered via the duodenal cannula, and, simultaneously, 200 μCi of L-[1,4,5-3H]leucine (354 mCi/mmol) of 0.9% NaCl was given via the intraperitoneal cannula. Duodenal lipid infusion at 450 pmol of fatty acid/h, including the lipid emulsion (Intralipid) providing 450 pmol of fatty acid/h. Two hours after initiating the lipid infusion, 1 mCi of L-[4,5-3H]leucine (40 to 60 Ci/mmol) in 0.5 ml of 0.9% NaCl was administered via the jugular vein and 1 h later the rats were killed by aortic puncture. Plasma albumin, lipoproteins, and apolipoproteins were isolated as described below. The radioactivity in albumin and lipoproteins was determined in washed trichloroacetic acid precipitates (11) and in apolipoproteins as described below. In addition, several tissues (heart, lungs, kidneys, and a 2-g sample of thigh muscle) were removed and the specific radioactivity of tissue protein was estimated. Tissues were homogenized in 6% perchloric acid, and the acid-insoluble residue recovered following centrifugation was washed four times with 15 volumes of 0.5% trichloroacetic acid solution containing 0.1% t-leucine. The washed residue was digested with 0.1 N NaOH and sampled for determination of protein (14) and radioactivity.

Relative Organ Contribution, Method I (Isolated Double Labeling)

For portal vein infusion, a 7-mm-long polyethylene cannula (0.35 mm outer diameter) attached to silicone rubber tubing (0.64 mm outer diameter) was inserted through a small incision in an ileal vein and advanced until the tip lay in the superior mesenteric vein. The duodenum and, in some animals, also the mesenteric lymph duct were cannulated with polyvinyl tubing (0.97 mm outer diameter) (15), with care being taken in the latter case to disrupt all observed accessory mesenteric lymph channels. All tubing exited the abdominal cavity through small stab wounds. The animals were allowed to recover for 16 to 20 h in restraining cages with continuous infusions of 10% glucose, 0.85% NaCl, 0.04% KCl (glucose/saline) into the duodenum at 2.3 ml/h, and 0.9% NaCl into the portal cannula at 0.4 ml/h. Then the duodenal glucose/saline infusion was replaced with a lipid emulsion (Intralipid) providing 450 μmol of fatty acid/h. Two hours after the start of the lipid infusion, 1 mCi of L-[1,4,5-3H]leucine (120 Ci/mmol) in 1 ml of Intralipid was administered via the duodenal cannula, and, simultaneously, 200 μCi of L-[1,4,5-3H]leucine (354 mCi/mmol) of 0.9% NaCl was given via the intraperitoneal cannula. Duodenal lipid infusion at 450 μmol of fatty acid/h, including the lipid in the radioactive dose, was continued, and the chylous lymph from the rats with lymph fistulae was collected in ice. Two to four hours after administration of the labeled leucine, blood was drawn by aortic puncture and the lymph and plasma apolipoproteins were isolated and counted as described below.

Relative Organ Contribution, Method II (Isolated Organs Versus Intact Rat)

Intact Rats—Animals were prepared with cannulae in the right jugular vein and duodenum as described above. The subsequent maintenance of the animals was as in Method I, including a 16- to 20-h recovery period followed by continuous duodenal lipid infusion. Two hours after initiating the lipid infusion, 1 mCi of L-[1,4,5-3H]leucine (40 to 60 Ci/mmol) in 0.5 ml of 0.9% NaCl was administered via the jugular cannula. Blood was drawn at 1 to 3 h after the administration of label, and plasma apolipoproteins were isolated and counted as described below.

Isolated Perfused Liver—Livers were perfused in situ by a procedure (16) modified slightly from that of Mortimore (17). The 70 ml of perfusing perfusate was composed of 35 ml of deionized rat blood and 35 ml of Krebs-Ringer bicarbonate buffer containing 70 mg of glucose, 1.4 g of bovine albumin (Fraction V, Armour), 8000 units of penicillin G, and 5 mg of streptomycin sulfate. Perfusate pH was maintained at pH 7.4 with NaHCO3 and flow rate was 1.8 ml/min/g of liver. Liver donor rats and blood donor rats in most experiments were fed stock diet and used without additional preparation. In several experiments, however, the liver donors were prepared the day before with duodenal cannulae and exposed to the same duodenal infusion schedule as the intact rats (see above). The livers were perfused 2 h after starting the duodenal branch infusion. In these same experiments, the blood donor rats were given 5 ml of Intralipid (1800 μmol of fatty acid) by gavage 1 h before blood was drawn. In all experiments, 1 mCi of L-[1,4,5-3H]leucine (40 to 60 Ci/mmol) in 1 ml of 0.9% NaCl was injected into the portal vein cannula 5 min after perfusate recycling was begun. Perfusate was sampled 2 to 4 h later and the lipoproteins and apolipoproteins were isolated and counted.

Isolated Intestine—Rats were prepared with duodenal and mesenteric lymph cannulae and maintained for 16 to 20 h as described above. Two hours after starting the duodenal lipid infusion, and under pentobarbital anesthesia, a segment of jejunum was isolated for study in vivo as previously described (12, 18). L-[1,4,5-3H]leucine (1 mCi, 40 to 60 Ci/mmol) in 0.3 ml of a balanced salt solution was injected into the lumen of a fat-absorbing segment from which all venous blood was collected, ensuring that only intestinal mucosa was exposed to the label. Labeled lipoproteins and apolipoproteins, unequivocally of intestinal origin, were isolated from the chylous lymph and venous blood collected from the preparation.

Isolation and Counting of Lipoproteins and Apolipoproteins

After addition of EDTA (0.1%) and NaCl (0.05%) to the lymph and to the blood or perfusate plasma, lipoprotein isolation from these samples was begun the same day by ultracentrifugation at 4°C. Total lipoproteins were isolated as a single fraction in most experiments. Samples were adjusted to d < 1.225 g/ml with solid KBr, overlaid with a KBr solution of d = 1.25 g/ml containing 0.85% NaCl and 0.1% EDTA, pH 7.4, centrifuged for 4.8 × 108 g-min in a Beckman SW-41 rotor, and the lipoproteins were recovered by tube slicing. Lipoproteins, without further washing, were dialyzed and partially delipidated with ethyl ether before apolipoproteins were separated by polyacrylamide gel electrophoresis and stained (12) (Fig. 1). Apolipoproteins in some samples were also separated by isoelectric focusing (12). Apolipoprotein bands were identified as described previously (12). It is apparent from Fig. 1 that lipoproteins isolated by this procedure show little contamination with other plasma proteins. In several experiments, plasma VLDL (d < 1.006 g/ml) and HDL (1.06 < d < 1.21 g/ml) were separately isolated by a modification of the density gradient procedure of Redgrave et al. (19). Samples of 4 ml or less, adjusted to 1.225 g/ml with KBr, were overlaid in sequence with equal vol-
Plasma Apolipoprotein Contributions by Liver and Intestine

RESULTS

Sources of Lipoproteins Other Than Liver and Intestine—One hour following an intravenous dose of \[^{3}H\]leucine, the specific radioactivity of plasma apolipoproteins in eviscerated, functionally hepatectomized rats was about 1% of that found in control animals; even this low incorporation of label may have been due to residual liver activity since albumin in the eviscerated animals was also slightly labeled (Table I). Consistent with this view, most of the lipoprotein label found in the eviscerated animals was in apo-E, the major hepatic apoprotein product (see below). The surgical trauma of evisceration, per se, apparently did not prevent other tissues from synthesizing apolipoproteins, since extensive incorporation of \[^{3}H\]leucine into the proteins of several tissues in the surgically treated rats was observed (Table I). The generally higher tissue specific activities in these animals compared to controls may have been due to the absence of hepatic and intestinal competition for the \[^{3}H\]leucine and also to the different dietary treatment of these animals during the preceding 20 h.

The possibility that spleen may synthesize apolipoproteins was not eliminated by these studies since splenic blood flow was also arrested by the surgery. This possibility seems remote, however. In the mathematical treatment of the leucine incorporation data to follow, it was assumed therefore that the plasma pool of newly synthesized apolipoproteins reflects the combined contributions from liver and intestine only.

Relative Organ Contribution, Method I (Differentiaaly Double Labeling)—In this approach, intestine was preferentially exposed to \[^{3}H\]leucine and liver to \[^{14}C\]leucine by the simultaneous administration of the \[^{3}H\] label intraduodenally and the \[^{14}C\] label intraportally. Thus, proteins derived from intestine would be expected to have a higher \[^{3}H]/[^{14}C\] ratio than those made by liver. This expectation was, indeed, realized (Table II). The r values shown are the \[^{3}H]/[^{14}C\] ratios normalized for the \[^{3}H]/[^{14}C\] ratio of albumin in the same animal. As expected, the actual \[^{3}H]/[^{14}C\] ratio found for albumin, 4.70 ± 0.22, was slightly lower than the ratio of administered radioactivity, 5.30 ± 0.05, since not all the intraduodenally administered \[^{3}H\]leucine reached the portal blood, a portion of it having been incorporated into protein by intestine. Since liver is the only organ known to synthesize albumin, any apoprotein having an r value of 1.00 can be assumed to derive only from the liver. The higher the observed r value, the larger the contribution by intestine.

Apoproteins made by intestine reach the blood mainly by way of mesenteric lymph (8, 12). In the rats with lymph fistulae, where most of the intestinal products were diverted, the r values of plasma apolipoproteins were, in fact, only slightly larger than 1.00 (Table II). Values larger than 1.00 reflect a residual intestinal contribution by way of accessory lymphatic-vascular anastomoses and the direct release of some intestinal apolipoprotein products into blood (8, 12). In mesenteric lymph, however, r values much larger than 1.00 were found for the three apolipoproteins shown previously (12) to be most actively synthesized by the intestine (Table II). The r values for all lymph apolipoproteins declined when lymph collection was extended beyond 2 h, reflecting an increased contribution from liver-derived plasma apoproteins (with r values of 1.00) filtered into the lymph. The diluting effect of filtered proteins on the r values of lymph proteins would be small during the first 2 h, the interval during which about 90% of the labeled apoproteins made in intestine follow-

### Table I

| Protein | Plasma proteins | Tissue proteins |
|---------|-----------------|----------------|
|         | Lipoproteins    | Albumin        |
|         |                 | Kidney         |
|         |                 | Lung           |
|         |                 | Muscle         |
| Normal control | 870 (46) | 280 (42) | 33 | 95 | 58 | 5 |
| Eviscerated, hepatectomized | 10 | 1 | 93 | 207 | 66 | 19 |

Per cent of normal

- Normal control: 1 (1.1)
- Eviscerated, hepatectomized: (0.4) (282) (218) (114) (380)

### Table II

| Protein | Plasma (dpm/µg protein) |
|---------|-------------------------|
| Albumin | 1.00 |
| Apo-A-I | 1.13 (0.08) | 4.73 | 3.40 | 3.28 | 1.98 (0.03) |
| Apo-A-IV | 1.63 (0.35) | 5.53 | 4.57 | 3.51 | 2.96 (0.07) |
| Apo-C | 1.17 (0.04) | 4.59 | 4.09 | 2.20 | 1.20 (0.08) |
| Apo-E | 1.04 (0.02) | 1.67 | 1.20 | 1.23 | 1.11 (0.02) |

| Protein | Rats with lymph fistula | Rats without lymph fistula |
|---------|-------------------------|---------------------------|
|         | 2–4 h 1 | 2 h 2 | 3 h 3 | 4 h 4 |
| Albumin | 1.00 | 1.00 |
| Apo-A-I | 1.13 (0.08) | 4.73 | 3.40 | 3.28 | 1.98 (0.03) |
| Apo-A-IV | 1.63 (0.35) | 5.53 | 4.57 | 3.51 | 2.96 (0.07) |
| Apo-C | 1.17 (0.04) | 4.59 | 4.09 | 2.20 | 1.20 (0.08) |
| Apo-E | 1.04 (0.02) | 1.67 | 1.20 | 1.23 | 1.11 (0.02) |

' Values are means (S.E.) of four rats killed at 2 to 4 h following label administration.

' Values are means from two rats; individual values differed from the mean by less than 10%.

" Single determination.
TABLE III

Contribution by liver and intestine to plasma apolipoproteins (Method I, differential double labeling)

For each plasma apolipoprotein, the per cent of total isotope contributed by intestine was calculated as follows:

\[
\text{% of } {}^3\text{H} \text{ by intestine} = \frac{4.96 (r - 1)}{r (4.95 - 4.9)} \times 100
\]

\[
\text{% of } {}^{14}\text{C} \text{ by intestine} = \frac{r - 1}{4.95 - 1} \times 100
\]

where \( r \) is the \( r \) value of the apolipoprotein in the plasma of the rats for derivation of equations). The per cent of total isotope contributed by liver equals 100 minus the per cent contributed by intestine.

| Apolipoprotein | Intestine | Liver |
|----------------|-----------|-------|
| {}^3\text{H}  | {}^{14}\text{C} | {}^3\text{H}  | {}^{14}\text{C} |
| Apo-A-I        | 62.0      | 24.8  | 38.0  | 75.2  |
| Apo-A-IV       | 83.2      | 49.6  | 16.6  | 50.4  |
| Apo-B          | 20.9      | 5.1   | 79.1  | 94.9  |
| Apo-C          | 12.4      | 2.2   | 87.6  | 97.8  |
| Apo-E          | 6.0       | 1.3   | 94.0  | 98.7  |

where \( r \) is the \( r \) value expected for a protein made exclusively in the intestine.

In rats without lymph fistulae, where the plasma reflects the contributions from both tissues, \( r \) values did not change significantly between 2 and 4 h and ranged from 1.05 for apo-E to 2.96 for apo-A-IV, revealing widely different relative contributions by intestine (Table II). From these values, and using 1.00 and 4.95 as \( r \) values for proteins made only in intestine and liver, respectively, one can calculate the intestinal and hepatic contribution of \( {}^3\text{H} \) and \( {}^{14}\text{C} \) for each apoprotein (Table III). Since the intestine was preferentially exposed to \( {}^3\text{H} \), the per cent intestinal contribution of \( {}^3\text{H} \) for each apolipoprotein was, as expected, always greater than its contribution of \( {}^{14}\text{C} \). Likewise, since the liver was preferentially exposed to \( {}^{14}\text{C} \), its contribution of this isotope was greater. Thus, the \( {}^3\text{H} \) data in Table III overestimate the intestinal apoprotein contribution while the \( {}^{14}\text{C} \) data underestimate it. The true apoprotein contribution from each organ, based on incorporation of circulating leucine, lies within the limits defined by the pairs of values given in Table III, e.g. intestine contributed between 25% and 62% of the apo-A-I but only 1 to 6% of the apo-E. A more precise estimate is precluded by uncertainty about the magnitude of preferential isotope exposure.

Relative Organ Contribution, Method II (Isolated Organs Versus Intact Rat)—In this approach, the patterns of apolipoprotein synthesis, i.e. the relative incorporation of \([{}^3\text{H}]\)leucine into the individual apolipoproteins, were first determined for isolated liver, for isolated intestine, and for the intact rat. The relative contribution of each organ needed to account for the pattern of synthesis observed in the intact animal was then calculated.

Perfused rat livers incorporated about 2% of the \([{}^3\text{H}]\)leucine dose into perfusate apolipoproteins (Fig. 2). Neither the total amount of apolipoprotein radioactivity nor its distribution among the individual apoproteins varied significantly between the 2nd and 4th h, although the perfusate was being continuously recirculated through the livers. This shows that there was no significant uptake of newly synthesized apoproteins and that the observed pattern of \([{}^3\text{H}]\) incorporation reflects the true synthetic pattern of liver under these conditions. Apolipoprotein analysis by isoelectric focusing confirmed the distribution of \([{}^3\text{H}]\) illustrated in Fig. 2 and, in addition, showed that of the total \([{}^3\text{H}]\) incorporated into apo-C, about 40%, 36%, 19%, and 6% was incorporated into apo-C-III-3, apo-C-III-1, apo-C-II, and apo-C-III-2(-1), respectively. The pattern of hepatic synthesis was similar when liver and blood donor rats were fed stock diet only and when liver and blood donor rats were fed large amounts of fat by duodenal infusion and gavage, respectively, during the 1- to 2-h period preceding surgery (see “Experimental Procedures”). The distribution of newly synthesized apolipoproteins among the perfusate VLDL and HDL is given in Table IV. Since HDL is the predominant lipoproteins in the perfusate and apolipoprotein exchange among lipoproteins is characteristic, it was not unexpected that most of the newly synthesized apo-C and apo-E were associated with HDL. All apo-A-I and apo-A-IV were also in HDL and virtually all apo-B in the VLDL.

The pattern of apolipoprotein synthesis by isolated intestine was established in earlier experiments (12) in which lymph and plasma VLDL and HDL were routinely separated and
individually washed by ultracentrifugation before apolipoproteins were analyzed. Additional similar experiments were therefore performed in which the total lipoproteins from lymph or plasma were isolated as one fraction by a single ultracentrifugation. The pattern of synthesis observed in these experiments did not differ significantly from that found previously, and the results from all the studies were combined. A mean of 90% of the newly synthesized apolipoproteins appeared in the mesenteric lymph and 10% in the venous blood collected from the isolated preparations. Release into blood averaged 7%, 16%, and 2% for newly synthesized apo-A-I, apo-A-IV, and apo-B, respectively.

Intact rats incorporated about 0.47 ± 0.02% of the \([^{3}H]\)leucine dose into total plasma lipoproteins in 1 h following the intrajugular administration of label. The distribution of \(^{3}H\) among plasma apolipoproteins changed gradually with time due to different turnover rates for the individual proteins (Fig. 3). Newly synthesized apo-B disappeared from the circulation most rapidly. The plasma distribution of radioactivity 1 h after \([^{3}H]\)leucine administration was chosen to reflect most reliably the relative rates of apoprotein synthesis in the intact animals; at 1 h, the release of labeled hepatic products would be virtually complete, the appearance of labeled intestinal products in blood via mesenteric lymph would be more than 85% complete,\(^2\) and the effect of unequal turnover rates would not be serious.

We considered the possibility that a pool of apoproteins in the plasma fraction of \(d > 1.21 \text{ g/mL}\) might exchange with the labeled apoproteins of intraluminally derived lipoproteins after the latter enter the blood, resulting in a differential loss of certain labeled apoproteins and a distortion of the \(^{3}H\) distribution pattern. To test for this possibility, an aliquot of labeled lymph from an isolated intestinal segment and the dialyzed total lipoprotein fraction from such lymph were each incubated with a dialyzed \(d > 1.21 \text{ g/mL}\) fraction from 2 volumes of unlabeled rat plasma and the total lipoproteins isolated. The distribution of \(^{3}H\) among the various labeled apolipoproteins was not changed significantly following either type of incubation.

The patterns of \([^{3}H]\)leucine incorporation are summarized in Table V. The major product in liver and the whole animal was apo-E while the major product in intestine was apo-A-I. The relative contribution of each organ needed to account for the incorporation pattern observed in the intact animals was then determined using the data in Table V. From multiple regression analysis, 80.9% of the \([^{3}H]\)leucine incorporated into the total plasma apolipoprotein pool \(in vivo\) was incorporated by liver and 19.1% by intestine. Relative incorporation rates of \([^{3}H]\)leucine do not necessarily reflect, however, the relative rates of apoprotein synthesis, since the specific radioactivities of the precursor leucine pools in the two tissues may have different. Adopting an approach suggested by the work of Scornik (21), we therefore compared the incorporation pattern in intact rats following the usual tracer dose of \([^{3}H]\)leucine with that following a massive dose, equivalent to more than 500 times the plasma leucine pool and several times larger than the total body pool. The massive dose would be expected to flood the tissues with labeled leucine from a common source and tend to equalize the specific radioactivities. The incorporation patterns observed following a tracer dose and a massive dose were not significantly different (Table VI). We could therefore reasonably conclude that 80.9% of the total plasma apolipoprotein pool was synthesized in liver and 19.1% in intestine.

### Table V

Patterns of \([^{3}H]\)leucine incorporation into apolipoproteins

| Apolipoprotein | Isolated liver | Isolated intestine | Intact rat, plasma |
|----------------|----------------|--------------------|-------------------|
|                | % total \(^{3}H\) | % total \(^{3}H\) | % total \(^{3}H\) |
| Apo-A-I        | 9.0 (0.3)       | 47.9 (3.8)         | 16.4 (0.4)        |
| Apo-A-IV       | 4.3 (0.2)       | 20.0 (3.7)         | 8.1 (0.4)         |
| Apo-B          | 25.0 (1.5)      | 20.0 (3.9)         | 25.0 (1.7)        |
| Apo-C          | 19.1 (0.9)      | 4.5 (0.5)          | 14.5 (0.6)        |
| Apo-E          | 42.6 (1.2)      | 1.5 (0.2)          | 36.1 (1.8)        |
| (Total)        | (100)           | (100)              | (100)             |

\(^a\) Values are means (S.E.) of 10 samples from 7 rats collected at 2 to 4 h after \([^{3}H]\)leucine administration.

\(^b\) Data include apolipoproteins released into mesenteric lymph and plasma during 3-h interval after \([^{3}H]\)leucine administration. Means (S.E.) of seven rats.

\(^c\) Samples were collected 1 h after \([^{3}H]\)leucine administration. Means (S.E.) of six rats.

### Table VI

Effect of leucine dose on the pattern of incorporation into plasma apolipoproteins by intact rats

Rats were prepared as described under "Experimental Procedures" (relative organ contribution, Method II, intact rats). The indicated dose of \(L-[^{4},^{5}]\)H-leucine (1.0 to 2.5 mmol) was given via the jugular vein cannula and plasma collected 2 h later. Apolipoproteins from total lipoproteins were isolated and the radioactivities were determined as described under "Experimental Procedures." Values are means (S.E.) for four animals. None of the difference between the two groups was significant (\(p > 0.05\)).

| Apolipoprotein | Leucine dose | % total \(^{3}H\) |
|----------------|--------------|------------------|
|                | 0.02 \(\mu\)mol | 720 \(\mu\)mol |
| Apo-A-I        | 10.2 (1.1)   | 19.9 (2.8)       |
| Apo-A-IV       | 6.5 (0.6)    | 8.5 (2.0)        |
| Apo-B          | 19.9 (2.0)   | 17.9 (2.1)       |
| Apo-C          | 13.9 (1.9)   | 14.5 (1.1)       |
| Apo-E          | 44.4 (2.6)   | 40.2 (3.8)       |
| (Total)        | (100)        | (100)            |

\(^2\) A.-L. Wu, unpublished observation.
The results show that more than 50% of the plasma apo-A-I and apo-A-IV in fat-fed rats had its origins in the small intestine. The possible importance of intestine as a source of apo-A-I was previously suggested from an estimate of lymph apo-A-I by Glickman and Green (23) and from an estimate of intestinal contribution to lymph apo-A-I by Imaizumi et al. (24). Apo-A-I and apo-A-IV in normal plasma are associated almost exclusively with HDL (3, 6). However, in mesenteric lymph, the route of most intestinal lipoproteins into the blood, a large portion of intestinally derived apo-A-I and apo-A-IV are associated with small and large chylomicrons (12). Transfer of apo-A-I to HDL is a rapid process dependent only in part on the metabolism of these triacylglycerol-rich lymph particles (25–27). No protein similar to apo-A-IV has been characterized as yet in human plasma, although such a protein has recently been found in human and also canine thoracic duct lymph (28), further strengthening the already substantial homology between apolipoproteins in rat and man. It is now evident that the intestine is an important source of plasma HDL protein.

Apo-A-II in the rat, unlike in man, is present as a monomeric and relatively minor apolipoprotein (5). In this study, insufficient quantities of apo-A-II were present on the polyacrylamide gels to permit a reliable assessment of organ contributions. Intestine, in addition to synthesizing more than 50% of the plasma apo-A-I and apo-A-IV, was the source for about 16% of the plasma apo-A-II. Apo-B from neither liver nor intestine has been well characterized, and it has, in fact, been suggested (29) that the apo-B from the two organs are not identical. Apo-B is thought to play a functional role in triacylglycerol transport from both organs, based on studies in humans with an inherited deficiency of this protein (30) and studies in rats fed orotic acid (31). It is interesting to note that the fraction of total apo-B derived from intestine and the fraction of total plasma triacylglycerol derived from intestine in rats on a fat-free diet (32) are about the same.

The C apoproteins as a group are synthesized largely in the liver, only about 5% being derived from the intestine. Most of the C apoproteins are well separated by isoelectric focusing (33), so the capacity to synthesize individual apo-C could be assessed in experiments with isolated intestinal segments (12) and isolated livers. The results revealed that intestine contributed about 10% of the total plasma apo-C-II, the activator protein for lipoprotein lipase in the rat (34), and about 7% of the apo-C-III-O. At least 99% of the apo-C-III-3 and 90% of the apo-C-III-2(-1), a very minor component in the rat, were derived from liver. The high pl of apo-C-I has complicated its analysis by isoelectric focusing. However, by the type of polyacrylamide gel electrophoresis-isoelectric focusing difference analysis discussed previously (12), apo-C-I also seemed to be derived almost exclusively from liver.

Apo-E, the arginine-rich protein, was the most actively synthesized of all the apoproteins, not only in the isolated liver, confirming the results of Marsh (9) and of Felker et al. (10), but in the whole animal (Table V). The values in Table V for relative incorporation of [3H]leucine into the various apolipoproteins in the intact rat are reasonably close approximations of the relative quantities of these proteins synthesized because (a) the similar patterns of incorporation observed with low and massive doses of labeled leucine (Table VI) indicate that even with a trace dose, proteins in liver and intestine are synthesized from a precursor pool of similar specific radioactivity; and (b) the leucine content of all the apolipoproteins is similar (6). Little or no apo-E was synthesized by intestine. A physiological role for this most
prominently synthesized apoprotein remains to be clearly established; it may serve to code lipoproteins for removal from the circulation by particular tissues (35).

The long-held view of conventionally isolated lipoproteins as lipid-protein particles that are synthesized, function in lipid transport, and finally are catabolized as more or less discrete entities seems no longer tenable. A more complex concept of lipoprotein origin and metabolism is evolving, involving participation by a number of tissues. Future application of the methods presently developed may help define the roles of liver and intestine in lipoprotein biogenesis under other physiological and dietary conditions. As data become available on the total turnover rate of individual plasma apolipoproteins, the absolute rate of apolipoprotein synthesis by each organ can also be determined.

Acknowledgments—We gratefully thank Mr. Albert E. Spaeth for his expert technical help and Dr. Mones Berman for help with the computer-assisted multiple regression analysis.

APPENDIX

Derivation of Equations for Calculating Relative Organ Contribution To Plasma Apolipoproteins, Method I
(Differential Double Labeling)

For any apolipoprotein, let \( a = \frac{^{1}H}{^{1}C} \) incorporated by intestine; \( b = \frac{^{14}C}{^{14}C} \) incorporated by liver; \( d = \frac{^{1}H}{^{1}H} \) incorporated by intestine; \( c = \frac{^{14}C}{^{14}C} \) incorporated by liver; \( r = \frac{^{3}H}{^{1}C} \) ratio for apolipoprotein divided by \( ^{3}H/^{14}C \) ratio for albumin; \( i = r \) value for all apolipoprotein made in intestine. Since all albumin is made in liver, its \( ^{3}H/^{14}C \) ratio is \( c/d \). The \( r \) value for all apolipoprotein made in liver is \( \frac{(c/d)}{(c/d)} \). The equation for \( i \) becomes

\[
i = \frac{a/b}{c/d} = \frac{ad}{bc}  
\]

(1)

\[
i - 1 = \frac{b(d - c)}{bc}  
\]

(2)

In plasma, where the contributions from liver and intestine are reflected,

\[
r = \frac{(a + c)/(b + d)}{c/d} = \frac{d(a + c)}{b + d}  
\]

(3)

\[
r - 1 = \frac{ad - bc}{bc + cd}  
\]

(4)

If \( x \) is the fraction of incorporated \( ^{1}H \) contributed by intestine, and \( y \) is the fraction of incorporated \( ^{14}C \) contributed by intestine, then

\[
x = \frac{a}{a + c}  
\]

(5)

\[
y = \frac{b}{b + d}  
\]

(6)

Multiplying Equations 5 and 6 by \( (r - 1)/(r - 1) \) and substituting from Equations 1 to 4:

\[
x = \frac{a(r - 1)}{(a + c)(r - 1)} = \frac{ad - bc}{bc + cd}  
\]

(7)

\[
y = \frac{b(r - 1)}{(b + d)(r - 1)} = \frac{bc(b + d)(r - 1)}{bc + cd}  
\]

(8)

Since the value of \( i \) was estimated to be 4.95 (see text)

\[
x = \frac{4.95(r - 1)}{(4.95 - 1)}  
\]

\[
y = \frac{r - 1}{4.95 - 1}  
\]

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