Identity of cells containing apolipoprotein B messenger RNA, in 6- to 12-week postfertilization human embryos

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Summary

Apolipoprotein B (Apo B) mRNA has been localized by in situ hybridization to various cell types in the liver, gut and yolk sack of the 6- to 12-week postfertilization human conceptus.

In the fetal liver it is probable that the immature hepatocytes contain Apo B mRNA. In the yolk sack, the Apo B cDNA probe hybridizes mainly to the large endodermal cells and in the fetal gut the epithelium seems responsible for the majority of Apo B mRNA production. The fetal brain did not show any detectable hybridization to the Apo B probe.

Unlike the situation seen in the adult, immunoprecipitation experiments demonstrated that only the B100 form of the protein was synthesized and secreted by the liver, gut and yolk sack at this early stage of human development.

Key words: apolipoprotein B, human embryo, in situ hybridization, lipid transport, gene expression.

Introduction

Many rapidly multiplying mammalian cells in culture require a source of lipids (e.g. Chen & Kandutsch, 1981); both in culture and in the adult body, this requirement can be satisfied by lipids which are delivered to the cell surface on lipoprotein particles (for recent review see Sparks & Sparks, 1985). It is likely that the cells of the newly implanted mammalian conceptus also need an exogenous lipid supply. Cell multiplication is particularly fast between 7 and 13 days, after fertilization in mouse development (e.g. Kauffmann, 1968; Snow, 1977), and a lipoprotein delivery system appears to develop very early: the mouse visceral endoderm is known to secrete the protein components of lipoprotein particles during the latter part of this period (Shi & Heath, 1984; Meehan, Barlow, Hill, Hogan & Hastie, 1984).

The growth of the cellular mass of the human conceptus is also very rapid soon after implantation, although there are no measurements of cell cycle duration (Moore, 1982). The aim of this paper is to describe the expression of the apolipoprotein B gene during the 6- to 12-week period of postfertilization human development; its product is the major protein of low density lipoprotein (LDL) particles and these particles probably transport lipids to the rapidly dividing cells of the human embryo.

It is not known to what extent the mother supplies the lipid needs of the conceptus. Preimplantation mammalian blastocysts have the capacity to synthesize lipids from simple precursors (reviewed in Pratt, 1982) and they may also be able to obtain lipids from the maternal circulation: the exterior cells of mouse blastocysts display coated pits and endosomes, which are necessary but not sufficient for ingesting lipids brought to the cell surface as apolipoprotein particles (Fleming & Pickering, 1985; Maro, Johnson, Pickering & Louvard, 1985; Pratt, 1985). Little is known about the balance between the endogenous synthesis of lipids and the external supply of lipids during the first three months of human development in the reproductive tract.

The expression of apolipoprotein genes and the secretion of their protein products has already been described in whole organs during the first and second trimester of human development (Shi, Hopkins, Thompson, Heath, Luke & Graham, 1985; Hopkins, Sharpe, Baralle & Graham, 1986; Zannis, Cole, Jackson, Kurnit & Karathanasis, 1985). Here, we have located the cells that contain high steady state levels of apolipoprotein B mRNA, by using the technique of in situ hybridization on sections of the
human conceptus. Consequently we map the gene expression that is characteristic of particular human embryonic cells. With this information and the additional study of the forms of apolipoprotein B secreted by embryonic and extraembryonic organs, we can compare the expression of the apolipoprotein B gene both during development and in the relatively well-studied adult.

Materials and methods

Embryo samples

Embryonic material was collected as described by Hyldahl (1984) and the postfertilization age was estimated as described by Thompson, Stern, Webb, Walsh, Engstrom, Evans, Shi, Hopkins & Graham (1984) and the material was processed within 1–4 h.

Metabolic labelling and immunoprecipitation

Metabolic labelling procedures were similar to those of Shi et al. (1985); in our work the incubation medium was Alpha MEM: methionine-free Eagle’s MEM in the ratio 1:10, with 1% (v/v) dialysed fetal calf serum, supplemented with [35S]methionine (Amer sham International plc, Amersham, UK, specific activity 1450 Ci.mM−1) at a concentration of 100 Ci.mL−1.

Apolipoprotein B was precipitated from the organ culture medium using a rabbit anti-human polyclonal antibody (Behring, supplied by Hoechst (UK) Ltd, Salisbury Rd, Hounslow, UK). The immunoprecipitation procedure was that described by Thompson et al. (1984) except that the precipitate was washed at pH 8.0 (S. Thompson, personal communication).

To separate the high molecular weight forms of apolipoprotein B, electrophoresis was performed on slab gels containing 3% (w/v) polyacrylamide (method of Weber & Osborn, 1969) or 5% (w/v) polyacrylamide gels (method of Laemmli, 1970). The gels were run at 30 mA for 18–20 h, or 35 mA for 4 h, respectively. The molecular weight markers were SDS molecular weight markers (MW SDS 200) and crosslinked phosphorylase b markers (P8906, both from Sigma, Poole, Dorset, UK). Adult LDL particles were prepared as described in Heath (1986). The mobility of the major protein species in these particles was taken as a marker for apolipoprotein B-100. The molecular mass of the labelled apolipoproteins was estimated by calculating a regression through the relative mobilities of the marker proteins.

The gels were stained with Coomassie blue, dried and the radioactivity in the gel was detected with preflashed Kodak X-Omat S X-ray film, using an intensifying screen. The film exposure period was 2–4 weeks at −70°C.

In situ hybridization

The protocol of P. Holland was followed with minor modifications (described in Hogan, Constantini & Lacy, 1986). During the preparation of the tissue, it was found that less air was trapped in the OCT embedding compound if the embedded samples were first frozen in liquid freon before plunging them into liquid nitrogen. The frozen samples were stored for up to 6 months at −70°C without any apparent degradation.

The sections were cut at 7 μm on a Slee cryostat and they adhered tightly to the slide without 0.25 M-ammonium acetate treatment prior to coating the slide with poly-l-lysine. There was no need to silic onize the coverslips. The slides were either stored immediately at −20°C with silica gel or they were dried, postfixed and dehydrated before storage. The two techniques gave similar tissue preservation and there was no obvious difference in hybridization efficiency.

The hybridization probes were anti-sense (hybridizes with apolipoprotein B coding mRNA) and sense (control) RNA’s. They were made from plasmids pApo B 1 and 2, by linearization at the EcoRI site in the poly linker and run off transcription from the SP6 promoters using [35S]UTP. The plasmids were constructed by cloning the 1.7 kb BamHI/HindIII fragment of an Apo B cDNA (Shoulders, Myant, Sidoli, Rodriguez, Cortese, Baralle & Cortese, 1985) into plasmid pSP62 (Melton, Krieg, Rebagli ati, Maniatis, Zinn & Green, 1984) in both orientations at the BamHI site. The specific activity of the [35S]-labelled UTP was 1000–1500 Ci.mM−1 (NEG 039H: NEN, Du Pont (UK) Ltd, Wedgewood Way, Stevenage, Herts, UK), and the total counts in the probe ranged from 2.3–8.6×106 cts min−1.

For the hybridization reaction on the slide, the probes were reduced in size to 50–150 base lengths by using the alkaline hydrolysis method of Cox, Deleon, Angerer & Angerer (1984). The size of the product of hydrolysis was checked on a 2% (w/v) agarose gel, and it was found that the hydrolysis period had to be extended to 3.5 h to achieve this length of probe. Each slide was reacted with 30 μl of hybridization buffer. In each experiment, both the probes contained identical counts: the total counts added to each slide, ranged from 0.5 to 1.7×106 cts min−1 in different experiments.

Slide washing and autoradiography followed the Holland protocol. The slides were not stained because the stain made it difficult to grain count under both phase- and dark-field optics.

Grain counting

Grains were counted in the autoradiographic emulsion, which was over and adjacent to parts of flat tissue sections, lying in a background of even grain density. A graticule with squares was placed in a ×8 eyepiece, and with a ×40 phase objective it was possible to count grains in 17×34 μm contiguous rectangles, which ran in a straight path from views of the slide alone into views of the tissue.

Northern and slot blots

The same cDNA probes were placed in the SP6 polymerase reaction with [α-32P]GTP (specific activity 800 Ci.mM−1, NEG 006X, NEN) and total counts of 3×106 to 1×107 cts min−1 were obtained. They were hybridized to Northern blots of the organs to verify both the specificity of the reaction observed on tissue sections and to measure the relative mRNA abundance in different organs.
The subsequent procedures were as described previously (Hopkins et al. 1986), with the addition of more stringent washing procedures to eliminate cross hybridization to ribosomal RNA. After the hybridization reaction, the blots were washed four times in 0.1xSSC with 0.1% (w/v) SDS at 65°C, each wash lasting 20 min. This was followed by incubation for 30 min at 37°C in RNase A (Sigma) at 10 μg ml⁻¹ in 0.5 M NaCl. The blots were then rinsed in 2×SSC at room temperature (RT), air dried, wrapped in Saran wrap (Dow Chemical Company, from Genetic Research Instrumentation Ltd, Takeley, Bishop’s Stortford, Herts, UK) and the radioactivity on the gel was detected and quantified as described before (Hopkins et al. 1986).

Results

In situ hybridization

We have previously observed abundant apolipoprotein B transcripts in gut, liver and yolk sack, using a denatured double-stranded cDNA probe on Northern blots (Hopkins et al. 1986). We also noticed that trace steady-state levels of the message could occasionally be found in the stomach, kidney, adrenal and brain. These trace amounts were always well below a 1% relative abundance per total RNA when compared to liver.

We first wished to establish that a single-stranded RNA probe hybridized with a similar apolipoprotein transcript. The anti-sense probe hybridized preferentially with slot blots (Fig. 1, see Materials and methods). This probe also hybridized with a large transcript of apparent size 11000 bases when compared to DNA size markers on Northern blots (results not shown: one liver at 63 days postfertilization, one gut at 71 days postfertilization). The organ specificity of this hybridization was similar to that observed previously (Fig. 1), except that the brain did not show a reaction at the level of sensitivity used in these experiments. The sense probe did not hybridize with any RNA transcripts, thus establishing that the RNA is only transcribed from one strand of DNA in the probed apolipoprotein B gene region.

A similar preferential hybridization was observed on sections. Grains were counted in transects, which ran from views of the slide alone to views of the neighbouring tissue section (called edge counts, Table 1, see Materials and methods). These grain counts showed that the liver and the secondary yolk sack sections reacted preferentially with the anti-sense probe, which should hybridize with apolipoprotein B mRNA. There was no evidence of a hybridization between these tissue sections and the sense probe. Qualitatively similar results were obtained with the gut and for each tissue three hybridization experiments were conducted with different probe preparations on different days and each experiment gave similar results.

![Fig. 1. Titration of the hybridization reaction, using total RNA from liver, gut and brain. Slot blots were hybridized with the anti-sense probe (closed symbols), which reacts with apolipoprotein B mRNA. The hybridization with the sense control probe is demonstrated by open symbols. The film was scanned to estimate the intensity of reaction with each probe. Liver (●, ○), gut (▲, △), and brain (◆, ◆).](image-url)
The brain did not show a preferential reaction with the anti-sense probe (Table 1). This result was also obtained after a 2-day exposure. After a 2-week exposure there was a slightly greater reaction with the anti-sense probe, amounting to about two grains per square. The inconsistency of this preferential reaction precludes any firm interpretation.

We attempted to identify the cell types which showed this preferential reaction. Grains were counted over regions of the sectioned organs, where different cell morphologies could be seen through the emulsion with phase optics (Table 2, Figs 2, 3). The thinnest profiles of the yolk sack wall were chosen for study, because these views were likely to be transverse sections of the wall. In the secondary yolk sack, the large 'endoderm' cells displayed a preferential reaction. Circular profiles of the gut villi were chosen for study and taken to be transverse sections of the villi. The lining epithelial cells around the gut lumen reacted strongly with the probe. The orientation and organ location of the liver sections could not be determined. There were strong hybridization signals from all the regions except the nests of nucleated haemopoietic cells. In all these organs, the rest of the cell types showed a minor preferential reaction. We believe that these minor hybridization signals are due both to the difficulty of recognizing rare strongly reacting cells with these optics and to the path distance of $^{35}$S radiation in the emulsion, which will tend to reduce grains in the emulsion at some distance from the site of hybridization.

In previous studies, we have observed low abundance apolipoprotein B transcripts in the stomach, kidney, adrenal and brain. It was possible that each of these organs contained rare cell populations which expressed high levels of this transcript. Despite careful grain counting of many brain sections and a thorough search through sections of the stomach and the kidney, we have been unable to detect such populations. They may have been missed because we did not serially section any of these organs. It is more likely that these rare apolipoprotein transcripts are evenly distributed through these organs, and that they do not show up in the grain counts.

**Molecular mass of secreted apolipoprotein B**

The adult liver and gut secrete different molecular mass forms of apolipoprotein B (see Discussion) and

### Table 1. Variation of grains counts over the slide and edge of tissue*

<table>
<thead>
<tr>
<th>Organ</th>
<th>Hours of exposure</th>
<th>Total no. edges counted</th>
<th>Average no. of grains/square over slide</th>
<th>Average no. of grains/square over tissue</th>
<th>Difference (grains over tissue minus grains over slide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yolk sack</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt.</td>
<td>20</td>
<td>11</td>
<td>0.91 ± 0.08</td>
<td>12.99 ± 0.82†</td>
<td>12.08</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>5</td>
<td>0.41 ± 0.09</td>
<td>0.40 ± 0.13†</td>
<td>-0.01</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt.</td>
<td>20</td>
<td>12</td>
<td>0.56 ± 0.07</td>
<td>6.82 ± 0.57†</td>
<td>6.26</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>6</td>
<td>0.87 ± 0.26</td>
<td>1.57 ± 0.19†</td>
<td>0.70</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt.</td>
<td>20</td>
<td>12</td>
<td>1.41 ± 0.14</td>
<td>2.11 ± 0.27</td>
<td>0.97</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>12</td>
<td>0.92 ± 0.11</td>
<td>1.77 ± 0.27</td>
<td>0.85</td>
</tr>
</tbody>
</table>

* Values are $\bar{x} \pm$ s.e.m. In most cases the edge counts were obtained from two to three sections.
† See Materials and methods section for an explanation of grain counting.

### Table 2. Variation in labelling between different cell types within an organ*,†

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cell type</th>
<th>Probe for message (anti-sense)</th>
<th>Probe for control (sense)</th>
<th>Difference (message−control)</th>
<th>No. of sections counted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yolk sack</td>
<td>Endoderm</td>
<td>12.99 ± 0.82</td>
<td>0.04 ± 0.13</td>
<td>12.59</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Mesenchyme</td>
<td>1.84 ± 0.39</td>
<td>0.47 ± 0.12</td>
<td>1.37</td>
<td>2</td>
</tr>
<tr>
<td>Liver</td>
<td>Hepatocytes</td>
<td>7.30 ± 0.70</td>
<td>1.20 ± 0.19</td>
<td>6.10</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Haematopoietic tissue</td>
<td>2.14 ± 0.42</td>
<td>0.31 ± 0.12</td>
<td>1.83</td>
<td>2</td>
</tr>
<tr>
<td>Gut</td>
<td>Epithelium</td>
<td>9.24 ± 1.38</td>
<td>0.53 ± 0.20</td>
<td>8.71</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mesenchyme</td>
<td>2.38 ± 0.60</td>
<td>0.37 ± 0.10</td>
<td>2.01</td>
<td>2</td>
</tr>
</tbody>
</table>

* Values are $\bar{x} \pm$ s.e.m., where $\bar{x}$ is the average grain count/square.
† All organs are 20 h exposures.
Fig. 2. Autoradiographs of the distribution of apolipoprotein B mRNA produced from in situ hybridization of human fetal tissues. Horizontal rows represent: yolk sack; gut and liver. The first photograph in these rows was taken using phase optics and the adjacent photograph was taken in the identical position using dark-field illumination and an oil immersion lens. The third photograph is the control taken under the same conditions as the second. The control photograph is taken from almost the same area as the experimental, the two regions being separated by one or two sections; consequently the control region does not represent the average grain count recorded in Table 1. Exposure times were all 20 h. Fetal ages are 76, 85, and 64 days postfertilization, respectively. m, mesenchyme; bv, blood vessel; en, endodermal cells; ep, lining epithelium; hm, haemopoietic tissue; h, hepatocytes. Scale bar, 20 μm.
so we characterized the metabolically labelled forms that were immunoprecipitated from the culture medium over gut, liver and secondary yolk sack.

The highest molecular mass form had a similar mobility to the major form obtained from adult LDL particles, which was known to have a molecular mass

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**Fig. 3.** The distribution of apolipoprotein B (Apo B) mRNA as determined by grain counting of autoradiographs produced from *in situ* hybridization of human fetal tissues. (A) yolk sack, (B) gut, (C) liver and (D) brain. The exposure times are all 20h. Fetal ages are 76, 85, 64 and 64 days, respectively. Each symbol represents the number of grains per rectangle. Open symbols are grain counts over the slide alone. Closed symbols are grain counts over the tissue section. In the case of the gut, the closed circles and triangles are counts over the lining epithelium, while the closed squares are counts over the internal mesenchyme of the villi. In the case of the liver, the closed circles and triangles are counts over the large epithelial cells, while the closed diamonds represent counts over the haemopoietic islands.
Apolipoprotein B mRNA location

89

Fig. 4. Electrophoresis of the forms of apolipoprotein B secreted by the secondary yolk sack and fetal liver. Electrophoresis of [35S]methionine-labelled material immunoprecipitated from the culture medium and run on a 5% SDS–PAGE slab gel. (A) Yolk sack, 39 days postfertilization. (B) Liver, 70 days postfertilization. The origin, the position of Apo B from adult LDL and the position of the molecular weight standards are indicated on the left.

of about $550 \times 10^3 M_r$ (Kane, Hardman & Paulus, 1980, see Fig. 4). This form was the predominant form detected over three gut samples (60 days postfertilization, two unknown age), one liver sample (70 days postfertilization), and two secondary yolk sack samples (39 and 44 days postfertilization). One yolk sack sample was run on two different 3% polyacrylamide gels and the estimated molecular mass was in the range 592–618 $\times 10^3 M_r$ (see Materials and methods).

Lower molecular mass forms were also found. The gut, liver and the secondary yolk sack each displayed a form that had a molecular mass in the range of 78–88% of the highest form. The secondary yolk sack also displayed two additional forms at 70% and 40% of the molecular mass of the highest form. There was no evidence of the B-48 form which is secreted by adult gut (see Discussion).

Discussion

Lipids are insoluble in aqueous solutions and they are transported around the adult body in association both with albumin and with apolipoproteins. Most apolipoproteins are transcribed from genes with similar structures (reviewed by Luo, Li, Moore & Chan, 1986). Apolipoprotein B gene structure has not yet been described, but it is already known to be an exceptional apolipoprotein gene with a very large transcript; a complete cDNA sequence has a length of 14 121 bases. (Knott, Pease, Powell, Wallis, Rall Jr, Innerarity, Blackhart, Taylor, Marcel, Milne, Johnson, Fuller, Lusis, McCarthy, Mahley, Levy-Wilson & Scott, 1986; Yang, Chen, Gianturco, Bradley, Sparrow, Tanimura, Li, Sparrow, Deloof, Rosseneu, Lee, Gu, Gotto Jr & Chan, 1986; Knott, Wallis, Powell, Pease, Lusis, Blackhart, McCarthy, Mahley, Levy-Wilson & Scott, 1986).

Synthesis of apolipoprotein B-100

Several forms of apolipoprotein B are found in the abundant lipoprotein particles which circulate around adult humans (reviewed Kane, 1983). Apolipoprotein B-100, B-74, and B-26 are mainly synthesized in the adult liver; their synthesis is presumed to be necessary for the formation of both low (LDL) and very low density (VLDL) lipoprotein particles, because such particles are absent from patients who lack these forms of apolipoprotein B (reviewed Herbert, Assmann, Gotto & Fredrickson, 1983; Herbert, Hyams, Bernier, Berman, Saritelli, Lynch, Nichols & Forte, 1985). Lipoprotein particles with the electrophoretic mobility of LDL particles have been found in the serum of a 29-day postfertilization embryo (Gitlin & Biasucci, 1969) and the liver of the second trimester fetus synthesizes and secretes particles which contain apolipoprotein B (Zannis, Kurnit & Breslow, 1982). Further, the fetal brain contains mRNA which can be translated into immunologically identified apolipoprotein B (Dziegielewksa, Saunders, Scheijjer, Zakut, Zevin-Sonkin, Zisling & Soreq, 1986).

It was previously known that during the first trimester of human development, the apolipoprotein B genes in the liver and the secondary yolk sack were transcribed and translated into an uncharacterized secreted form of apolipoprotein B (Shi et al. 1985; Hopkins et al. 1986). This study establishes that the molecular mass of the predominant apolipoprotein B secreted by the gut, the liver and the secondary yolk sack is sufficiently similar in mobility to that of adult LDL particles to regard these forms as embryonic equivalents of the $550 \times 10^3 M_r$ or B-100 form described by Kane et al. (1980). The mobility of this predominant embryonic form relative to adult LDL and our estimate of its molecular mass, suggest that it
may have a slightly higher molecular mass in the conceptus. The lower embryonic molecular mass forms appear to have different mobilities to those described in adult LDL particles; this makes it difficult to assess their adult equivalents. In the 21-day postfertilization fetal rat, Apo B-100 is the only form synthesized by the yolk sack and placenta, and it is the predominant form which is synthesized by the liver (Demmer, Levin, Elovson, Reuben, Lusis & Gordon, 1986).

The LDL particles have many functions in adult lipid metabolism (reviewed by Mahley, Innerarity, Rall & Weisgraber, 1984; Sparks & Sparks, 1985). There are also hints that they may function in early mammalian development: LDL particles partly support the multiplication of undifferentiated human teratoma cells and mouse embryonal carcinoma cells and their derivatives in culture (Engstrom, Rees & Heath, 1985; Heath & Deller, 1983; Rizzino, 1983). LDL particles are also necessary for the sustained and rapid synthesis of steroids by the human fetal adrenal, the fetal testis and placental cells in culture (Carr, Parker, Milewich, Porter, MacDonald & Simpson, 1980; Carr, Parker, Ohashi, MacDonald & Simpson, 1983; Winkel, Snyder, MacDonald & Simpson, 1980). It is probable that these functions are met by the local synthesis of apolipoprotein B at the earliest stages of mammalian embryogenesis. There is evidence that this protein is expressed in 11- to 12-day postfertilization mouse visceral yolk sack (Shi & Heath, 1984; Meehan et al. 1984).

Lack of detectable apolipoprotein B-48 synthesis
Apolipoprotein B-48 is found in chylomicrons; these are produced in the adult gut and the bulk of apolipoprotein B-48 is believed to be synthesized here in adult humans (reviewed by Bisgaier & Glickman, 1983; Kane, 1983). Chylomicrons are not synthesized by individuals that lack apolipoprotein B-48, and it is therefore assumed that apolipoprotein B-48 synthesis is required for chylomicron production. The chylomicrons are secreted into the adult mesenteric lymph and they are one of the vehicles for transporting dietary triglycerides and fat soluble vitamins from the gut to the rest of the body. We have been unable to detect the synthesis of the apolipoprotein B-48 form by the embryonic gut. This observation may indicate that chylomicrons are not produced by the gut during this period of development. Such an interpretation is not improbable, because there will be little 'dietary' lipid in the gut lumen during this period; the duodenum is a blocked tube for much of this time (Moore, 1982). If the gut is not an absorptive organ during this period, then the function of gut apolipoprotein B-100 is unclear. During the fetal development of the rat intestine, there are marked variations in Apo B mRNA abundance and by 21 days of postfertilization development the predominant synthesized form is Apo B-48 (Demmer et al. 1986). It is therefore probable that the human gut will show different patterns of Apo B gene expression in the second and third trimester of development.

Cellular distribution of apolipoprotein B transcripts
It is probable that the synthesis of serum proteins starts early in secondary yolk sack development: alphafetoprotein, albumin and transferrin can be detected inside the large endoderm cells at 20 days after fertilization (immunohistochemistry: Krag Jacobsen, Jacobsen & Boll Henriksen, 1981). These cells have an extensive endoplasmic reticulum at this time (Hesseldahl & Falck Larsen, 1969). 50 days postfertilization is the earliest age at which there is direct evidence for the synthesis and the secretion of apolipoprotein B by the yolk sack (Shi et al. 1985). We have found high concentrations of apolipoprotein B transcripts in the endoderm cells and this is a direct demonstration that it is these cells that are the source of the secreted apolipoprotein B.

There is uncertainty about the exact time at which the human embryonic liver begins to secrete serum proteins. Metabolically labelled transferrin, $\alpha_1$-antitrypsin, $\alpha_2$-macroglobulin and prealbumin has been detected in culture medium over livers taken at 29 and 32 days after fertilization (Gitlin & Biasucci, 1969). It is not until 42 days after fertilization that there is direct evidence for apolipoprotein B synthesis and secretion by the liver (Shi et al. 1985). It is difficult to identify the cells in the embryonic liver that contain apolipoprotein B gene transcripts. During the 6th and 7th weeks of postfertilization development, the hepatic sinusoids are not formed and the most obvious cellular formations are the nests of nucleated haemopoietic cells. The RNA in the cells of these nests does not display a strong hybridization reaction with the apolipoprotein B probe. The large epithelial cells in the remaining tissue consist of immature hepatocytes, bile duct progenitor cells, and immature blood cells (Holmes, Davis, McCarthy & Stirrat, 1986; Holmes, personal communication). We have been unable to identify which of these cell types reacts with the probe. It is probable that it is the immature hepatocytes, because it is these cells in the adult rat liver that exhibit apolipoprotein B determinants in the endoplasmic reticulum (Alexander, Hamilton & Havel, 1976). It should also be noted that hepatocytes display very variable amounts of intracellular serum proteins even after the hepatic sinusoids have been formed (Glieberman & Abelev, 1985).

It is known that most of the embryonic endoderm cells of the 20-day postfertilization conceptus neither
possess a brush border nor contain intracellular serum proteins (Krag Jacobsen et al. 1981). The gut does not develop a highly folded internal surface until 7 to 8 weeks after fertilization (reviewed Grand, Watkins & Torti, 1976). At this period, the internal surface of the epithelial cells becomes covered with microvilli and the cells have an extensive endoplasmic reticulum (Kelley, 1973). The earliest age at which there is direct evidence for apolipoprotein B synthesis and secretion by the gut is 56 days (Shi et al. 1981). The gut that reacts with the probe directly establishes that these cells are the source of this serum protein. These cells retain this function through to the adult, since they synthesize and secrete apolipoprotein B when cultured in isolation from the other cells of the adult gut (Rachmilowitz, Sharon & Eisenberg, 1980).

The cellular distribution of apolipoprotein B transcripts emphasizes the similar functions of some liver cells, the gut epithelium and the endoderm layer of the secondary yolk sac. Most other kinds of apolipoprotein genes are expressed in a much wider variety of organs in human development (Hopkins et al. 1986; Zannis et al. 1985).

This work was supported by the Cancer Research Campaign and the British Heart Foundation. We are grateful to Dr Christopher Holmes and Dr James Scott for advice and access to unpublished results, and Dr Valerie Tate for comments on the manuscript.

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Apolipoprotein B mRNA location 91


(Accepted 15 January 1987)