Transferrin in foetal and adult mouse tissues: synthesis, storage and secretion

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SUMMARY
Transferrin is an important growth-promoting serum glycoprotein synthesized chiefly in the liver in adults. The transferrin found in the mouse foetus is thought to be wholly a product of the foetus itself and its synthesis starts at least as early as the 7th day of gestation. The major sites of synthesis in mouse foetuses are the visceral yolk sac (VYS) and liver (Adamson, 1983). We now report that other murine foetal tissues synthesize readily detectable amounts, namely lung, spleen, spinal cord and rib cage. Very low levels are also synthesized by the brain, muscle and pancreas. We can detect no synthesis of transferrin in late foetal thymus, heart or skin although mid-gestation foetal skin may make a very small amount. No synthesis of transferrin can be detected in adult brain, lung and spleen, but approximately equal rates of synthesis are detected in adult liver and adult ear pinna.

Transferrin is accumulated by foetal and adult tissues in widely varying amounts and these have been measured by enzyme-linked immunosorbent assays of extracts. In addition to VYS and liver, high levels of transferrin are found in foetal skin, lung and rib cage with lower amounts in spinal cord, spleen and muscle tissues. Tissues of the 15th day foetus accumulate the highest concentrations of transferrin. A role for the mediation of transferrin in the stimulation of growth and differentiation by interacting tissues is discussed.

INTRODUCTION
Transferrin is a β1-globulin glycoprotein (M_r = 80 000) present in serum at 2–4 mg ml^-1 (Putnam, 1975), and capable of tightly binding two ferric and other metal ions. Its principal function is regarded as the transport of iron, and its targets are those tissues which are actively growing such as embryonal and tumour cells and those cells with special iron requirements such as haematopoietic cells. Iron (and zinc) is required for all dividing cells to maintain enzyme and co-factor levels in new cells. Transferrin is known to be adsorbed to cells through a specific receptor glycoprotein (M_r = 180 000) and is probably endocytosed into vesicles called receptorosomes. From here an acidic compartment removes iron and this is found later in the cytosol, bound to ferritin. Transferrin, unlike most ligands which enter via receptors, appears to be recycled out of the cell and reutilized in the extracellular fluid (reviewed by Octave, Schneider, Trouet & Crichton, 1983).

Embryonal cells have special demands for transferrin for the synthesis of iron-containing haem and enzymes. Since there appears to be a barrier to the passage of

Key words: Mouse, transferrin, glycoprotein, liver, yolk sac.
transferrin from the maternal circulation (Faulk & Galbraith, 1979) at least up to the 18th day of gestation (Renfree & McLaren, 1974), it follows that transferrin should be an early product of the murine embryo. The earliest time that transferrin synthesis has been detected (Adamson, 1982) is on the 7th day of gestation. The endoderm layer of the two-layered egg cylinder appears to be the source and later this layer develops into the endoderm of the visceral yolk sac (VYS). The VYS and the foetal liver are major sources of transferrin during gestation. Both of these endodermally derived tissues also synthesize alphafoetoprotein (Dziadek & Adamson, 1978), but only transferrin continues to be synthesized by the liver in adults.

It is not yet known if transferrin is synthesized by any other foetal mouse organs, an important question in view of the ability of transferrin to stimulate the proliferation and differentiation of mouse foetal kidney (Ekblom et al. 1983), chick muscle (Markelonis, Oh, Eldefrawi & Guth, 1982a; Ii, Kimura & Ozawa, 1982; Beach, Popiela & Festoff, 1983), and lymphocyte growth in vitro (Brock, 1981; Anderson, Chase & Tomasi, 1982). Transferrin is an essential component of serum-free media (Sato et al. 1979) for all kinds of cell types. We have therefore made a survey of murine foetal tissues to identify those that synthesize transferrin and which may stimulate their own development or the development of associated tissues.

We have analysed the content of transferrin in several adult and foetal organs in order to identify transferrin which may be sequestered or accumulated to significantly high levels. The hypothesis is that transferrin could be released locally or preferentially to influence the development of associated tissues. These data together with transferrin synthesis rates are assessed to determine the possibility of a role for transferrin in tissue interactions which are known to stimulate organogenesis or cell proliferation.

**MATERIALS AND METHODS**

_Metabolic labelling and extraction of foetal tissues_

Random bred mice (CDI Flow Laboratories) were mated and the day of finding the copulation plug was designated the first day of gestation. Pregnant females were killed by cervical dislocation, the uteri removed and foetuses dissected to collect the tissues. Care was taken to remove skin and brain samples before opening the body cavity and dissections were made so that the liver fragments did not contaminate any other tissue. Tissues (0.05–0.1 g wet weight) were washed in Trypsin-EDTA for 10–30 min and twice in medium (DME with 10% foetal bovine serum) before pre-incubating them in 0.5 ml DME containing 1/20 normal concentration of methionine and 10% dialysed foetal bovine serum. After 30 min, [35S]methionine (100 μCi ml⁻¹, 1.2 Ci μmol⁻¹, New England Nuclear), in fresh medium was added and incubation continued at 37°C in 7% CO₂ in air. After 3 h, tissues were separated from the incubation medium, and the latter was centrifuged at 15000 g for 10 min at 4°C. Tissues were washed once in phosphate-buffered saline and homogenized in 0.5 ml hypotonic buffer. Lysing buffer contained 10 mM-KCl, 1 mM-MgCl₂, pH 7.5, 0.1 mM-PMSF, and after 15 min at 0°C, Triton X-100 was added to 0.5%. After centrifugation at 15000 g for 10 min the supernatant was removed for analysis and the pellet was dissolved in 0.5 ml 0.5 N-NaOH to assay for protein by the Coomassie blue-dye-binding procedure (Sedmak & Grossberg, 1977).

The medium and extracts were analysed as shown in the flow diagram (Fig. 1) for protein, total and acid-insoluble radioactivity, total transferrin content by enzyme-linked immunosorbent assay (ELISA) (see below) and for immunoprecipitated radioactive transferrin (see below).
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Fig. 1 Flow diagram to show foetal tissue extraction and analysis procedures.

Analysis of radioactive protein

From the medium and extracts prepared as above, 2 µl aliquots were examined for total radioactive protein by precipitation on filter paper squares with ice-cold 10% (w/v) trichloracetic acid (TCA) (30 min at 0°C). The squares were washed with cold 10% TCA containing 1 mM-methionine, followed by ethanol. They were dried and counted in 3 ml Beta-Max (West Chem Products, San Diego, CA) in a scintillation counter.

Appropriate aliquots of medium and extract were taken to contain 2 × 10^5 and 4 × 10^5 c.p.m. TCA-insoluble radioactivity, respectively. Aliquots of medium were made up to 1 to 1.2 ml with NET buffer (0.4 M-NaCl, 5 mM-EDTA, 0.05 M-Tris-HCl pH 8, 1% NP-40, 0.1 mM-PMSF and 0.02% sodium azide) and one portion was treated with 5 µl (approx. 5 µg) affinity-purified anti-transferrin (see below) while to another, 5 µl non-immune rabbit immunoglobulin was added. After incubation at room temperature for 1/2 h or overnight at 4°C, 30 µl of a 50% suspension of protein A-agarose (Sigma) was added and the mixture mixed end-over-end for 1/2 h at 4°C. The beads containing adsorbed antibody/antigen complexes were washed twice in NET buffer and once in 0.0625 M-Tris-HCl pH 6.8 before being boiled in 30 µl two-fold-concentrated gel sample buffer (Laemmli, 1970). Tissue extracts were analysed similarly except that the diluting and washing medium was RIPA buffer (0.01 M-Tris–HCl, pH 7.5, 0.15 M-NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulphate). Radioactive proteins were analysed by electrophoresis on 7.5% polyacrylamide gels (Laemmli, 1970), stained with 0.2% (w/v) Coomassie brilliant blue, destained and soaked in fluor (Autofluor, National Diagnostics, Somerville, NJ), dried and exposed to preflashed Kodak XAR-5 film for 5–21 days at −70°C (Bonner & Laskey, 1974). The bands on films were analysed by scanning densitometry and quantitated by integration of the peaks.

Analysis of transferrin by ELISA

Pooled mouse plasma was processed by the method described by Sawatzki, Anselstetter & Kubanek (1981) to isolate pure transferrin. Briefly, a 45% saturated-ammonium-sulphate-supernatant fraction from mouse serum was salted out on a column of Sepharose CL-6B, and the transferrin peak was rechromatographed on DEAE-Sepharose. Peak fractions from this column were homogeneous by several criteria (Adamson, 1982). Transferrin (200 µg) dissolved in PBS was mixed with an equal volume of Freund’s complete adjuvant and injected into several subcutaneous sites in one rabbit. Immunizations were repeated at 4-week intervals using Freund’s incomplete adjuvant. Serum from the third bleed onwards contained anti-transferrin activity in
double-diffusion tests. Antibodies were affinity purified by passing a fraction which was precipitated by 40% saturated ammonium sulphate over a conjugate of 50 mg purified transferrin linked to cyanogen-bromide-activated Sepharose (10 ml). After washing away unadsorbed protein, antibodies were eluted with 0.1 M-glycine- HCl, pH 2.6. Fractions with the highest precipitating activity were pooled, dialysed against 0.05 M-NH₄HCO₃ solution and lyophilized. The antibody was shown to react specifically with mouse transferrin by double-diffusion analyses (Adamson, 1982) and by lack of crossreaction with whole mouse, calf and rabbit sera in ELISA.

A portion (1 mg) of affinity-purified anti-transferrin antibodies was crosslinked to 5 mg alkaline phosphatase (bovine intestine, Sigma type VII) according to the method described by Engvall (1980) and this was used as the third layer in a sandwich ELISA. The wells of 96-well polystyrene plates (Linbro/Titertek, cat. no. 76-38104, Flow Laboratories, Inc., McLean, VA) were coated with affinity-purified rabbit anti-mouse transferrin (100 μl of 5 μg. ml⁻¹ antibodies dissolved in 0.1 M-sodium bicarbonate buffer at pH 9.5). This was allowed to adsorb for 16 h at room temperature. The plate was washed three times with 0.9% (w/v) NaCl, 0.05% (v/v) Tween 20 after each layer. The second layer was a solution (100 μl) containing an unknown or standard transferrin concentration from 2 to 200 ng. ml⁻¹ in PBS, 5% calf serum, 0.05% Tween-20 (Bovine transferrin does not react with the anti-mouse transferrin antibodies, Adamson, 1982, and therefore transferrin in calf serum does not interfere in the assay). Incubation and washing followed as described above. Rabbit antimouse transferrin coupled to alkaline phosphatase (200 μl of 0.5 μg. ml⁻¹) was added to each well for 5 h at room temperature and finally, 250 μl substrate for alkaline phosphatase (Sigma 104 at 1 mg. ml⁻¹ in 0.1 M-diethanolamine-HCl, 1 mM-MgCl₂, pH 9.8) allowed the enzyme activity in each well to be revealed as a yellow colour. The absorption at 405 nm was measured in a Flow titertek 96-well reader after 20 min and 30 min of colour development. Figure 2 shows a typical standard curve used to measure the concentration of transferrin in tissue extracts. The lower limit of sensitivity is 10 ng. ml⁻¹ transferrin.

**Measurement of transferrin in adult tissues**

Adult mice were perfused by passing 0.9% NaCl solution into the heart of anaesthetized heparin-injected animals using a 20 cm pressure head. Tissues were dissected and approximately 1 gm was homogenized and processed as described for foetal tissues.

![Fig. 2 Standard curve for transferrin ELISA. The concentrations of transferrin in individual wells (averages of duplicates) of a 96-well plate are shown on the abscissa (log scale). The optical densities of the products of alkaline phosphatase-linked (third layer) anti-transferrin antibodies is shown on the ordinate.](image)
RESULTS

Transferrin synthesis by foetal tissues

Figure 3A shows that large amounts of transferrin are synthesized by the liver and VYS of foetuses. Smaller amounts of radiolabelled transferrin are secreted into the medium by spinal cord (including ganglia), spleen, rib cage and lung. Some transferrin is also synthesized by the brain, skeletal muscle and pancreas. In general, transferrin synthesized at very low levels is detected best in the medium after the 3h incubation period, while the tissue extract contains much less. Transferrin synthesis was undetectable in 19th day thymus, heart and skin. Several experiments gave varying results and these are given separately in Table 1. Variations may arise in part because of differences in the exact stage of development between litters and in part by differential resistance to damage during processing. Some tissues of 15th day foetuses were also examined. Low levels of transferrin synthesis were detected in the spinal cord, brain and skin with large amounts in VYS incubates or 15th day tissues (Table 1).

It is clear that several foetal tissues can synthesize and release transferrin into the medium. However, examination of Coomassie-blue-stained gels showed that synthesis of transferrin may be of only minor importance compared to its storage or retention by certain tissues. Figure 4 shows that the skin of 19-day foetuses secretes sufficiently high levels of both alphafoetoprotein (lane 1) and transferrin (lane 2) after immunoprecipitation to stain as distinct protein bands on gels.

Table 1. Detection of transferrin synthesis in foetal tissues by metabolic labelling

<table>
<thead>
<tr>
<th>Tissue</th>
<th>15-day foetus</th>
<th>19-day foetus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>medium</td>
<td>tissue*</td>
</tr>
<tr>
<td>VYS</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>liver</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S.C.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>brain</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>thymus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>spleen</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>rib cage</td>
<td>±, +, +</td>
<td>±, +, +</td>
</tr>
<tr>
<td>muscle</td>
<td>+, −, ±</td>
<td>+, −, ±</td>
</tr>
<tr>
<td>heart</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>pancreas</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>lung</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>skin</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

VYS = Visceral yolk sac
S.C. = Spinal cord

* Tissue and incubation media were analysed separately for immunoprecipitated transferrin.
† For extracts that gave variable results, individual experiments are displayed between commas. Blank spaces indicate the tissue was not examined. The number of + signs indicates approximate quantitation by densitometry.
Similarly, spinal cord (lane 4), brain (lane 6), ribs (lane 11), muscle (lane 13) and heart (lane 15), in addition to VYS (lane 8) and liver (lane 10) release readily detectable transferrin into the medium during the 3 h incubation period. We therefore measured the transferrin content of various foetal tissues at several stages.

Transferrin content of foetal and adult tissues
To establish the size of the compartment of retained transferrin, total transferrin
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in tissue extracts and incubation media was measured by ELISA. These values are expressed as \( \mu g \) transferrin \( \cdot mg^{-1} \) protein in Figure 5 and as \( \mu g \cdot g^{-1} \) wet weight of tissue in Table 2. Among tissues of the 13th day foetuses, the placenta holds the

![Image](image_url)

**Fig. 4** Coomassie-blue-stained polyacrylamide gel showing the products analysed after immunoprecipitation of 19th day foetal tissues. Lanes 1–10 are the same as Figure 3A; lane 11, ribs, anti-transferrin; lane 12, skeletal muscle, control; lane 13, skeletal muscle, anti-transferrin; lane 14, heart, control; lane 15, heart, anti-transferrin. \( M_r \) markers are shown on the left and the positions of transferrin (TF) and alphafoetoprotein (AFP) on the right. Specifically immunoprecipitated protein bands are indicated by spots on the left hand side.

![Image](image_url)

**Fig. 3** Autofluorographs of immunoprecipitated metabolically labelled extracts. \([^{35}S]methionine\) was incorporated into tissues in organ culture for 3 h and media or tissues were immunoprecipitated with anti-alphafoetoprotein, anti-transferrin or preimmune rabbit control Ig as described in Materials and Methods. (A) Immunoprecipitated products from the medium of 19th day foetal tissues: lane 1, skin, anti-alphafoetoprotein; lane 2, skin, anti-transferrin; lane 3, spinal cord, control; lane 4, spinal cord, anti-transferrin; lane 5, brain, control (this precipitate appears to have missed the washing steps but serves to indicate the range of total biosynthesized proteins); lane 6, brain, anti-transferrin; lane 7, VYS, anti-alphafoetoprotein; lane 8, VYS, anti-transferrin; lane 9, liver, anti-alphafoetoprotein; lane 10, liver, anti-transferrin. (B) From 19th day foetal tissues: lane 1, lung, anti-transferrin; Lane 2, lung, control; lane 3, spleen, anti-transferrin; lane 4, spleen, control; lane 5, pancreas, anti-transferrin; lane 6, pancreas, control. (C) Immunoprecipitated products from the medium of 18th day foetal tissues: lane 1, spinal cord, control; lane 2, spinal cord, anti-transferrin; lane 3, VYS, anti-transferrin; lane 4, liver, anti-transferrin; lane 5, ribs, anti-transferrin. (D) adult tissues: lane 1, ears, control; lane 2, ears, anti-transferrin; lane 3, liver, control; lane 4, liver, anti-transferrin. The positions of relative molecular mass \( (M_r) \) markers are shown on the left and transferrin (TF) and alphafoetoprotein (AFP) on the right. Lower \( M_r \) bands in the heavy immunoprecipitates obtained from VYS and liver are likely to be breakdown products in these tissues; the higher \( M_r \) band seen in (B), lanes 5 and 6 and in (C), lane 5, is fibronectin which is specifically adsorbed by protein A. Exposures were for 14–38 days.
Fig. 5 Transferrin content of foetal and adult tissues. Transferrin was assayed in tissue extracts by ELISA and is expressed as μg . mg⁻¹ tissue protein. Conditions are given in Table 2. Tissues are: M, muscle or limb-bud; V, visceral yolk sac; L, liver, R, ribs; B, brain; S, skin; H, heart; Lu, lungs; S.C., spinal cord; K, kidney; P, placenta; T, thymus; Sp, spleen; Pa, pancreas; S.N., sciatic nerve; S.G., salivary gland; Ts, testes.

greatest store of transferrin, expressed as μg . mg⁻¹ protein. Considering the large size of the placenta compared to all other tissues, the importance of this store is clear. Up to the 15th day of gestation, the VYS appears to be predominant over the liver, but later in gestation the two tissues become equally important sources of transferrin.

Comparisons of the transferrin content of tissues dissected from 13th day, 15th day and 19th day foetus and adult perfused tissues show (Figure 5 and Table 2): (1) Tissues of the 15th day foetus, namely VYS, liver, spinal cord, brain, vertebrae or ribs, limbs and heart, accumulate higher concentrations of transferrin than any other stage examined. In all tissues except lung, peak levels of transferrin are found
Table 2. Transferrin content of washed foetal and perfused adult mouse tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>15th day foetus µg.g⁻¹ wet wt.</th>
<th>19th day foetus µg.g⁻¹ wet wt.</th>
<th>adult µg.g⁻¹ wet wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>VYS liver</td>
<td>100 ± 22</td>
<td>775 ± 625</td>
<td>352 ± 224</td>
</tr>
<tr>
<td>S.C. brain</td>
<td>14 ± 8</td>
<td>49 (5–173)</td>
<td>28</td>
</tr>
<tr>
<td>S.N. thymus</td>
<td>27 ± 2</td>
<td>29 ± 9</td>
<td>143 ± 101</td>
</tr>
<tr>
<td>S.N. spleen</td>
<td>43-3</td>
<td>63</td>
<td>170 (35–285)</td>
</tr>
<tr>
<td>rib cage</td>
<td>92-3</td>
<td>110 ± 82</td>
<td>114 ± 19</td>
</tr>
<tr>
<td>muscle heart</td>
<td>9-2</td>
<td>71 ± 16</td>
<td>145 ± 36</td>
</tr>
<tr>
<td>pancreas kidney</td>
<td></td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>lung sal. gl.</td>
<td>11-5</td>
<td>128 (41–215)</td>
<td>359 ± 71</td>
</tr>
<tr>
<td>testes skin</td>
<td>15-3</td>
<td>406 ± 232</td>
<td></td>
</tr>
</tbody>
</table>

The transferrin content of extracts was estimated by ELISA (see Methods section for details). Washed or perfused tissues were incubated for 3 h in culture media before separate analyses of total transferrin that was present in the medium and in the tissue. These figures were also used to derive those in Figure 5. VYS, visceral yolk sac; S.C., spinal cord; S.N., sciatic nerve; sal. gl., submandibulary salivary gland.

Means of 2–4 experiments ± standard deviations with at least triplicate samples. In some cases, parentheses showing the range are given. Single value indicates the result of one experiment with at least duplicate samples. Blank spaces indicate no determinations.

in the 15th day foetus; (2) In the lung, transferrin continues to accumulate throughout gestation and into the adult stage where levels become similar to the stores in liver; (3) Nervous tissues (spinal cord and brain) of the foetus accumulate transferrin to moderate levels which decline after the 15th day. The adult sciatic nerve contains considerable concentrations of transferrin; in some individuals this level is higher than in the liver when expressed as a proportion of the total protein concentration in that tissue; (4) The total store of transferrin in skeletal muscle is very high in adults and late foetuses considering that a high proportion of body mass is muscle. In addition, 15th day limb (predominantly developing muscle and cartilage) contains the highest concentrations of transferrin of any tissues examined. The rib cage and vertebrae of mid- to late-gestation mouse foetuses also contain high levels.

Release of transferrin by foetal tissues

We made a further comparison to determine the rates at which tissues could release transferrin into the medium during organ culture. Figure 6 shows that most
Fig. 6 Transferrin released by dissected tissues incubated in organ culture for 3 h at 37 °C. Transferrin was determined by ELISA as described in the Materials and Methods section. Tissues are those listed in Figure 5; data given in Figure 5 were obtained in the same experiments.

15th day foetal tissues (muscle/limbs, ribs/vertebrae, brain, skin and heart) are capable of releasing transferrin into the incubation medium at rates higher than that of liver. Similar observations hold for 19th day foetal muscle, rib cage, brain, skin, heart, lung and kidney. Very slow rates of release are detected from spinal cord, thymus, spleen, and pancreas when expressed as μg released mg⁻¹ tissue protein.

Transferrin synthesis by adult tissues

In addition to foetal liver and VYS, mouse spinal cord, brain, skin, lung, spleen, rib cage and pancreas in foetuses from the 15th to the 19th day of gestation synthesize transferrin as shown by metabolic labelling and electrophoretic analysis of immunoprecipitates (Fig 3A,B,C and Table 1). If we are to conclude that these sources of newly synthesized transferrin are significant for the development or maturation of tissues, we might expect that they are switched off in adult tissues when they would no longer be needed. Adult mouse lung, spleen, brain, liver and pinna of the ear (skin and cartilage, etc.) were labelled with [³⁵S]methionine as described in Materials and Methods. Autofluorographs show that synthesis of
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Transferrin is undetectable in lung, spleen and brain, but is occurring at approximately equal rates (expressed as c.p.m. g⁻¹ wet weight) both in liver and in ear pinna (Fig. 3D). The latter is a surprising and rich source that could derive from the sebaceous glands, epidermis, connective tissue, cartilage or blood elements contained within the pinna. Further studies will be needed to answer this question.

DISCUSSION

Synthesis of transferrin

We report here that transferrin may be synthesized by a variety of cell types in the foetal mouse, since several tissues secrete a metabolically labelled polypeptide that is antigenically related to adult mouse transferrin. It has previously been reported that several adult mammalian tissues (liver, leucocytes, macrophages, Sertoli cells, salivary gland, testis, ovary and lactating mammary gland; Thorbecke et al. 1973; Imrie & Mueller, 1968; Tormey, Imrie & Mueller, 1972; Haurani, Meyer & O'Brien, 1973; Skinner & Griswold, 1980 and 1983) synthesize transferrin but no foetal tissues other than liver and visceral endoderm have been identified to have this ability in human (Gitlin & Perricelli, 1970), rat (Yeoh & Morgan, 1974) and mouse foetuses (Adamson, 1982). Recently, however, it has been reported that chick embryo spinal cord synthesizes transferrin (Stamatos, Squicciarini & Fine, 1983). We find that midgestation (15th day) foetal spinal cord, brain and skin synthesize low but detectable levels of transferrin while very large amounts are produced by the VYS and liver (Table 1). Late foetal spinal cord continues to synthesize transferrin as do several other tissues (Fig. 3). The list of foetal tissues that synthesize transferrin may be much larger since it is possible that our detection system is insufficiently sensitive or that tissues are damaged during trypsin–EDTA or other treatments. Of those adult tissues examined, only the liver and the pinna of the ear synthesize readily detectable amounts (Fig. 3D). These results suggest that multiple synthetic sources of transferrin are required for the special demands of certain foetal tissues during gestation.

Importance of the visceral yolk sac

The VYS has long been known to play an important role in acting as a protective barrier, as a transporter of selected molecules as well as producing nutritive materials to the developing foetus. Transferrin is one of several nutritive macromolecules synthesized very early in mouse development (7th day) by the visceral endoderm and later by the VYS (Adamson, 1982). Other VYS products with carrier and nutritive roles are AFP (Yeoh & Morgan, 1974; Dziadek & Adamson, 1978) and apolipoprotein (Shi & Heath, 1984). The major source of transferrin for the rapidly proliferating cells of the developing embryo is clearly the VYS, since the foetal liver may only commence significant synthesis by the 13th day or later (15th day in rat, Yeoh & Morgan, 1974). The immense importance of the VYS in supporting embryonic development cannot be overstated. It therefore may seem
that other tissues that synthesize transferrin must be relatively minor sources. However, the proximity of a transferrin source or delivery by a special mechanism may be important for some developing tissues especially since arteries and blood capillaries would not yet be organized in the primordial organ buds of the embryo.

Retention of transferrin by tissues

In perfused tissues, most of the serum transferrin has been removed and what remains is present in these compartments: (1) Intracellular transferrin newly synthesized in that tissue; (2) Transferrin that has been endocytosed or that is bound to cell surface receptors; (3) Transferrin in the interstitial fluid and remaining serum. All of the compartments may release transferrin readily to the surrounding medium. The time taken for intracellular transferrin to be released after synthesis is less than 1½ h (our unpublished observations) and it has been documented that endocytosed transferrin may be released intact within minutes of entry into cells (Octave, Schneider, Trouet & Crichton, 1983). In these studies we have measured the release of transferrin from these combined compartments in order to estimate the influence that each tissue may have on nearby target tissues in terms of providing an essential nutrient or stimulator, namely, transferrin.

Transferrin is found in foetuses at levels far in excess of those in adults, therefore we conclude that transferrin is as necessary to embryonic development in general as it is to cells cultured in defined media. Transferrin is present in 15-day foetal tissues, at a time when rapid organ growth is occurring, at two- to fifty-fold higher levels than in 13th or 19th day foetuses. These findings indicate (a) possible specialized roles in certain tissues such as muscle, bone and skin, and (b) the importance of a store of transferrin at stages when the circulatory system is not well developed.

A developmental role for transferrin

Transferrin is a required factor for the proper development of muscle (Cohen & Fischbach, 1977; Podlewski et al. 1978; Kuromi, Gono & Hasegawa, 1981; Markelonis et al. 1982, 1982a; Stamatos et al. 1983; Matsuda, Spector & Strohman, 1984a; Matsuda, Spector, Micou-Eastwood & Strohman, 1984b), kidney (Ekblom, Thesleff, Miettinen & Saxen, 1981; Ekblom et al. 1983), teeth (Partanen, Thesleff & Ekblom, 1984) and blood (Broxmeyer et al. 1980; Pelus, Broxmeyer, de Sousa & Moore, 1981; Gentile & Broxmeyer, 1983). We show here that transferrin levels are highest when the foetal tissues' proliferative demands are very great and conclude that developmental requirements could be met by one or all of the following: (a) synthesis in the individual tissue; (b) retention and reuse; (c) delivery from adjacent tissues; (d) delivery by nerves.

There is some evidence that transferrin exerts a mitogenic effect independently of its ability to supply iron to proliferating tissues (Imrie & Mueller, 1968; Tormey et al. 1972). The recent findings that there is some homology between the oncogene Blym and transferrin (Goubin et al. 1983) also support the notion that transferrin may be a general mitogen.
Transferrin synthesis and secretion

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