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A fetuin-related glycoprotein (α_2HS) in human embryonic and fetal development

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Summary. The human plasma protein, α_2HS glycoprotein, has an amino acid composition very similar to that of fetuin, the major protein in fetal calf and lamb serum. Immunohistochemical studies of human fetuses (6–33 weeks gestation) showed that α_2HS glycoprotein and fetuin have similar distributions in developing brain and several other tissues, e.g., bone, kidney, gonads, gastrointestinal tract, respiratory and cardiovascular systems. There were notable differences in the liver and thymus in the distribution of the two proteins. Fetuin and α_2HS glycoprotein are present in plasma and cerebrospinal fluid of both human and sheep fetuses; their concentrations are reciprocally related: in human plasma and cerebrospinal fluid α_2HS glycoprotein concentration is high and fetuin low; the reverse is the case in sheep fetuses.

Estimates of the concentration of α_2HS glycoprotein in human fetal cerebrospinal fluid and plasma were obtained. It is suggested that α_2HS glycoprotein may play a role in developing tissues, especially in the human fetus, similar to that of fetuin in other species.

Key words: Brain, vertebrate – Development, ontogenetic – Proteins, plasma – Cerebrospinal fluid – Fetuin – α_2HS glycoprotein – Human – Sheep

The first fetal plasma protein to be described was fetuin; Pedersen (1944) purified this α_2-glycoprotein from newborn and fetal calf serum. It has subsequently been shown to be quantitatively important in the fetus of members of the Order Artiodactyla (cattle, sheep, pigs and goats, see Cavanagh et al. 1982 for references). It contributes up to 5 g/L or more in plasma throughout the long gestational period of these species, but is detectable in the adult only at a low concentration (e.g. 60 mg/L in sheep; Dziegielewska et al. 1980). Its function are not known, although there have been several suggestions (see Discussion).

Within the developing forebrain in species of the Order Artiodactyla there is a particularly striking immunocytochemical staining for fetuin in a high proportion of cells of the cortical plate when it first appears (30–35 days gestation in the sheep, Møllgård et al. 1984; Reynolds and Møllgård 1985; 26–31 days in the pig, Cavanagh and Møllgård 1985; 45–50 days in the cow, Reynolds et al. 1986). Fetuin also occurs within cells of many other embryonic and fetal tissues of sheep and pigs (Dziegielewska et al. 1981; Reynolds et al. 1983; Reynolds and Møllgård 1985; Cavanagh and Møllgård 1985). Until recently it was somewhat curious that such an apparently important glycoprotein appeared to be absent from other species. However, it has since been shown by the use of cross-reacting anti-fetuin antisera, that fetuin or a fetuin-like protein is present in the fetuses of many non-Artiodactyla species, including human and rodent (Dziegielewska et al. 1983). As will be described in this paper, some anti-fetuin antibodies stain the early cortical plate of human fetal brain but only very weakly and the amount of fetuin in human fetal plasma appears to be rather small. We have therefore investigated the possibility that another glycoprotein might have a distribution in human fetal brain and other tissues similar to that of fetuin in sheep, pig and cattle fetuses. Comparison of published values for amino acid compositions of human plasma proteins (e.g. Schultze and Heremans 1966) with the known composition of cattle (Spiro and Spiro 1962) and sheep (Marti et al. 1973) fetuins suggested that α_2HS glycoprotein (α_2HS, named after its co-discoverers J.F. Heremans and K. Schmid, see Putnam 1984 for review of properties) was a good candidate for the human equivalent of fetuin.

This study shows that both α_2HS and fetuin are present in at least some species, that there is a degree of cross reactivity between anti-fetuin and α_2HS, but that there are also some differences in the distributon of these proteins. A major finding is that, whereas fetuin is the main plasma protein present in early cortical plate cells in fetuses of Artiodactyla, it is α_2HS which is found in these cells at a similar stage of brain development in the human fetus.

Materials and methods

Tissues. Tissues examined in the immunocytochemical part of the study were obtained from 6 human embryos and 14 human fetuses selected from a group of 141 human embryos and fetuses obtained from legal abortions, ectopic pregnancies and stillborn fetuses. The embryos and fetuses ranged from 6 mm crown-rump length (CRL) to 44 cm total length corresponding to 6–33 weeks gestation.

Sheep embryos and fetuses were obtained from Clun Forest ewes of known date of mating. This part of the material, including handling, fixation, preparation and im-

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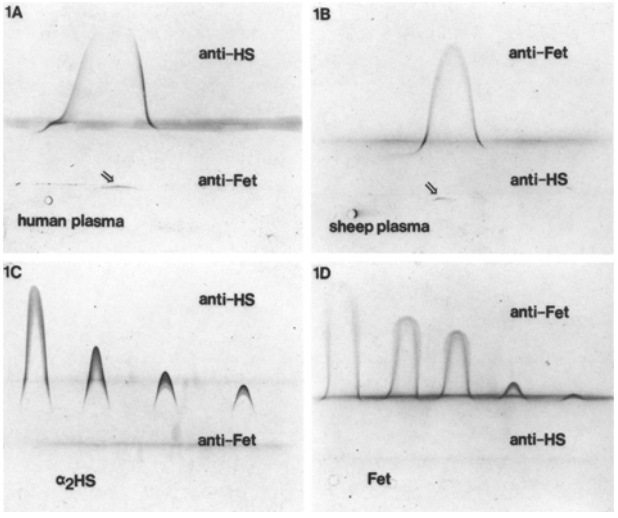


Fig. 1. Crossed immunoelectrophoretic (**A, B**) and rocket immunoelectrophoretic (**C, D**) plates. In **A** the intermediate gel contained anti-cattle fetuin (0.1 ml) and the top gel contained anti-human α_2HS (0.15 ml). The well contained 5 µl of 19-week human fetal plasma. Note the small peak for fetuin (*arrow*) and the much larger, stronger peak for α_2HS (top gel). In **B** the intermediate gel contained anti-human α_2HS (0.1 ml) and the top gel contained anti-cattle fetuin (0.2 ml). 5 µl of 40 day sheep plasma were placed in the well. Note small peak for α_2HS in intermediate gel (*arrow*) and much larger peak for fetuin in top gel. **A, B** clearly indicate the presence of both α_2HS and fetuin in human and sheep fetuses. In **C** the intermediate gel contained anti-fetuin (0.7 ml in 3 ml buffered agarose) and the top gel contained anti-α_2HS (0.15 ml in 4 ml buffered agarose). The wells in the bottom gel contained dilutions of α_2HS standard (500 µg/ml, left to 10 µg/ml, right). In **D** the intermediate gel contained anti-α_2HS (0.5 ml in 3 ml agarose) and the top gel contained anti-fetuin (0.2 ml in 4 ml agarose). The wells in the bottom gel contained dilutions of fetuin standard (100 µg/ml, left to 5 µg/ml, right). The clear extension of the immunoprecipitation lines into the intermediate gel in **C** is evidence of cross reactivity between α_2HS and anti-fetuin

munocytochemical staining, was described in detail recently (see Reynolds and Møllgård 1985). The methods used were similar to those described below for the human fetal material; only Bouin's fixative was used for the sheep fetal material.

Plasma and CSF samples. CSF (cisternal or ventricular) and plasma (derived by heart puncture) samples were obtained from human fetuses after legal therapeutic abortion, with the approval of the University College Hospital Ethical Committee. Samples were frozen as soon as possible after they were obtained and stored at −20° C until used. Samples of CSF and plasma from fetal sheep were obtained from the fetuses of anaesthetized ewes and stored at −20° C as described previously (Dziegielewska et al. 1980). Plasma proteins, including fetuin and α_2HS appear to be stable under the conditions used in the experiments.

Immunochemistry. Crossed immunoelectrophoresis as described previously (Dziegielewska et al. 1983) was used to identify fetuin and α_2HS in human and sheep samples. Individual details are given in the legend to Fig. 1. Rocket immunoelectrophoresis was carried out according to the description of Weeke (1973); details are again given in the legend to Fig. 1. Quantitative estimates of α_2HS in human CSF and plasma samples were obtained by radial immunodiffusion (Mancini et al. 1965). The standard used was Hoechst (FRG) Human Standard Serum Code No. ORDT 0203. The antiserum was Hoechst anti-α_2HS.

Antisera and specificity checks. Specific rabbit antiserum against bovine fetuin (code no. Z 249, lot 012A) was obtained from DAKOPATTS, Denmark, and rabbit antiserum against human α_2HS (104110A) was obtained from Hoechst (FRG). Working dilutions used in the immunocytochemical part of the study were determined by titration. Control tests with these antisera absorbed with purified sheep fetuin or with human α_2HS respectively, were negative. Both the polyclonal antibodies to fetuin and α_2HS and the monoclonal antibodies to fetuin (see below) recognize protein epitopes rather than the oligosaccharide.

Absorption tests showed a significant amount of cross reactivity between α_2HS and fetuin. Therefore in order to test whether α_2HS could be detected in tissues shown to stain for fetuin, advantage was taken of the fact, confirmed in control experiments, that the rabbit IgG-PAP system does not react with mouse monoclonal antibodies when incubated on tissue sections. Purified sheep fetuin was available from an earlier study (Dziegielewska et al. 1980). Monoclonal antibodies were raised by the standard hybridoma technique (Köhler and Milstein 1975). Ovine and bovine forebrain sections were pre-incubated with mouse monoclonal antibodies to sheep fetuin; they were then incubated with rabbit antisera to either ovine or bovine fetuin or to human α_2HS and developed with the usual rabbit PAP-DAB procedure. Appropriate controls were run with each test. Sections preincubated with monoclonal anti-fetuin were negative after incubation with anti-fetuin antisera but positive with anti-α_2HS antisera. Controls were negative. This demonstrates that specific α_2HS staining could be distinguished from cross reactivity between anti-α_2HS and fetuin.

Immunocytochemistry – fixation and preparation. Immediately after operation, the entire embryos or tissue blocks from the forebrains, which were removed from the fetuses, were fixed for 24 h at 4° C in one of the following fixatives: 10% buffered formalin, 4% formol-calcium, Lillie's fixative or Bouin's fixative. The tissues were dehydrated in ethanol and xylene at room temperature and embedded in paraffin at 58–60° C. Serial sections of 3–5 µm were cut in horizontal, sagittal or frontal planes. Two sections from every 10 were selected for histological examination and stained with toluidine blue or haematoxylin-eosin. Other serial sections from these brains, together with serial sections cut from stored paraffin blocks, were used for immunocytochemical tests. In a few cases both original and new serial sections were used and in these instances no differences in the staining reactions were demonstrated.

Immunoperoxidase staining. Paraffin sections were dewaxed and endogenous peroxidase activity was blocked with a fresh 0.5% solution of hydrogen peroxide in methanol for 15 min. Sections were subsequently rehydrated and washed 3 times for 10 min in 0.5 M NaCl in 0.05 µM Tris/HCl (Sigma) buffer, pH 7.4 to which 0.01% v/v Nonidet P-40 has been added (wash buffer). Thereafter, the sections were exposed to 20% v/v normal swine serum in 0.5 M NaCl, 0.05 M Tris/HCl (Sigma), pH 7.4 (dilution buffer)

at room temperature for 15 min. Excess swine serum was removed without washing and the sections were treated with either rabbit anti-bovine fetuin (1:200 in dilution buffer) or rabbit anti-human α_2HS (1:1500) overnight at 4° C. After incubation with antibody the sections were washed 3 times for 5 min at room temperature with wash buffer. The sections were treated with swine anti-rabbit immunoglobulin (1:20 in dilution buffer) for 30 min at room temperature. After washing 3 times 5 min, the sections were treated with a soluble complex of horseradish peroxidase and rabbit anti-horseradish peroxidase (PAP, DAKO-PATTS) 1:50 in dilution buffer followed by three times 5 min washes. The bound antibodies were visualized with a solution of 0.05% DAB (Sigma) in 0.05 M Tris/HCl (Sigma) pH 7.4 in 0.5 M NaCl. The sections were then pre-incubated for 10 min in this solution before incubation for 10 min in the same solution containing 0.05% H_2O_2. The DAB stained sections were washed for 5 min in Tris buffer, counterstained with haematoxylin, dehydrated and mounted in DPX.

Controls were included in each test; specific antisera were omitted or substituted by rabbit pre-immune serum in the same dilution as used for the antisera. Sections so stained were always negative although background staining varied: tests with high background were discarded. The specificity of the antisera was verified by testing on formalin-fixed and paraffin-embedded material with known positive reaction for the antigens.

Results

Immunochemistry

The presence of both fetuin and α_2HS in 19 week fetal plasma and 40 day sheep fetal CSF is illustrated in Fig. 1. In the human material α_2HS was much more prominent than fetuin (Fig. 1A) whereas in sheep there was far more fetuin than α_2HS. In both species α_2HS had a slower electrophoretic mobility than fetuin. Fetuin in human plasma samples has previously been reported (Dziegielewska et al. 1983) and appears to be present at least between the ages of 16 and 30 weeks gestation. Samples earlier than 16 weeks have not so far been available, but immunocytochemical staining of plasma fetuin in sections of embryos as young as 7 weeks was observed (see below). Because of the scarcity of human fetal CSF, we were unable to look for the presence of fetuin in this fluid, but it could be visualised immunocytochemically in sections of forebrain from fetuses in the age range 7–20 weeks. Quantitative estimates for α_2HS in human fetal plasma (Table 1) and CSF were obtained for a small number of samples. There was no significant difference in α_2HS concentration in plasma at the different fetal ages. The adult value is about 50–60 mg/100 ml (Putnam 1984). In fetal CSF (18–22 weeks) the α_2HS concentration was 4.3 ± 0.8 mg/100 ml ($n=6$), which is about 25 times higher than in the adult (Schuller et al. 1971). The CSF:plasma ratio was $13.7 \pm 2.5\%$ for the six paired samples; in the adult it has been reported to be 0.36% (Felgenhauer 1974). Quantitative estimates of fetuin in human fetal plasma and CSF could not be made because of the lack of a suitable human fetuin standard. By comparison with sheep samples for which a fetuin standard has been prepared (Dziegielewska et al. 1980) the amount can be estimated approximately to be 1–5 mg/100 ml at 19 weeks and less than 1 mg/100 ml at 30 weeks in plasma.

Table 1. Concentration of α_2HS (mean $\pm$ S.E.M.) in human fetal plasma between 18 and 40 weeks gestation; n = number of fetuses sampled

Fetal age (weeks)	n	α_2HS concentration (mg/100 ml)
20 (18–22)	9	31 ± 4
30 (26–34)	6	37 ± 7
38 (35–40)	4	47 ± 7

α_2HS in the sheep has not been previously described. It was detectable in trace amounts in adult plasma (not illustrated). An example of the detection of α_2HS in fetal sheep plasma (40 days) is shown in Fig. 1. With the methods used α_2HS could not be detected in CSF from 60 days gestation onwards. It was present at a low level in plasma over a wide period of gestation. Immunocytochemically it was detected over the age range 25–80 days. As with fetuin in the human fetus, we were not able to estimate the amount of α_2HS in fetal sheep plasma, because of the lack of a suitable standard. However, crossed immunoelectrophoretic data suggest that it was below 1 mg/100 ml. Thus fetuin is a major plasma and CSF protein in fetal sheep but α_2HS is present in only small amounts. In contrast, the opposite is the case in the human fetus; however in this species α_2HS is not nearly so quantitatively dominant in the plasma as fetuin is in the sheep.

The immunoelectrophoretic evidence given above shows clearly that both proteins are present in sheep and human fetuses. By varying the relative concentrations of antibody and antigen it was possible to demonstrate cross reactivity between α_2HS and anti-fetuin using rocket immunoelectrophoresis but not between fetuin and anti-α_2HS. An example is shown in Fig. 1C, D.

Immunocytochemistry

The distribution of α_2HS and fetuin in the lateral wall of the human cerebral vesicle prior to and during cortical plate formation. The terminology used to describe the different stages of the development of the neocortex was based upon the modifications of the Boulder Committee Scheme (1970) and proposals by Marin-Padilla (1978) which were used in the studies of human (Møllgård and Jacobsen 1984) and sheep (Reynolds and Møllgård 1985) cortex development. Neither α_2HS nor fetuin could be demonstrated in the ventricular zone of an embryo of 6 mm CRL but some cells positive for α_2HS were seen lining the ventricle in embryos of 9–15 mm CRL. A few scattered cells in the primordial plexiform layer also exhibited positive staining reaction for α_2HS. At later embryonic stages (CRL 16–22 mm) staining for α_2HS was seen in some cells in the primordial plexiform layer (Fig. 2). Fetuin was barely detectable but weak positive staining was distributed in a pattern similar to that of α_2HS.

By 22–24 mm CRL, when the cortical plate has developed as a layer of 2–5 cells thick in the lateral cerebral wall, many cells exhibited a distinct positive staining reaction for α_2HS (Fig. 3A). Irregularly orientated positive cells

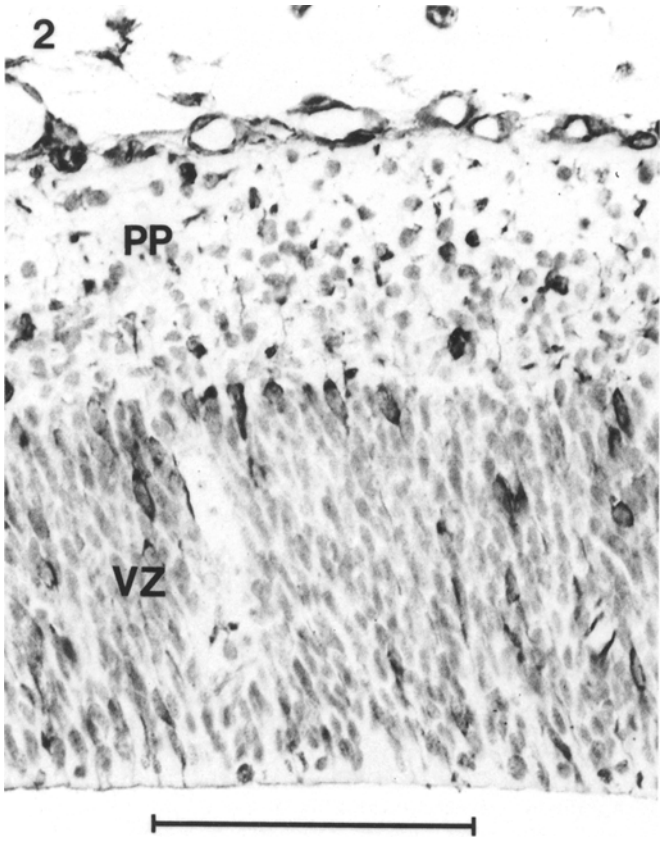


Fig. 2. Immunoperoxidase staining of a 20 mm CRL human embryo for α_2HS showing the characteristic staining pattern with positive cells distributed mainly in the outer half of the ventricular zone (*VZ*) and in the primordial plexiform layer (*PP*). Bar indicates 100 μm

were scattered in the well-defined subventricular zone. Very few positive cells could be identified in the ventricular zone. Fetuin staining showed a similar but much weaker pattern of reactivity (Fig. 3 B).

At later stages of neocortical development most of the well-differentiated cells located in the inner half of the cortical plate exhibited a positive staining reaction for α_2HS. Some of the large well-differentiated neurons in the subplate zone were also positive for α_2HS. A very weak staining reaction for fetuin could be demonstrated in the regions positive for α_2HS. In the oldest fetuses invesigated the staining reactions for both α_2HS and fetuin had disappeared.

The distribution of fetuin and α_2HS in the lateral wall of the sheep cerebral vesicle prior to and during the formation of the cortical plate. The distribution of fetuin-positive cells during the development of the sheep neocortex has been described recently (Reynolds and Møllgård 1985). The distribution of fetuin-staining in sheep embryos of 25 and 31 day gestation was very similar to that of the α_2HS-staining in human embryos (CRL 16–22 mm) as shown in Fig. 2. Fetuin-staining of the early cortical plate in 35–36 days sheep embryos was very similar to α_2HS-staining of 22–24 mm CRL (7 weeks) human embryos and fetuin-staining of early human cortical plate was very similar to α_2HS-staining of early sheep cortical plate.

This paper presents examples of the distribution of fetuin and α_2HS in different human tissues. To be able to compare the intensity of the staining reactions, dilutions similar to those used for staining the developing cortex (α_2HS 1:1500, fetuin 1:200) have been used throughout the study.

Other human tissues

Bone forming tissues. Since previous studies have emphasized an association between α_2HS and bone formation we have been particularly concerned to compare the distribution of α_2HS and fetuin in bone-forming tissues. Osteoblasts and osteoid material were positive for both proteins. The staining reaction for α_2HS was somewhat stronger than that for fetuin, but the distribution was practically identical (see Figs. 4 A, B).

Kidney and gonads. Both the intensity of staining and the distribution of positively stained cells in the kidney were very similar (Fig. 5 A, B). Intraluminal material stained very similarly along the length of the tubules. Many proximal tubular cells were positively stained for both proteins, but there was also a proportion of cells in the loop of Henle, distal tubule, and collecting tubule stained similarly for fetuin and α_2HS.

The gonads showed a very strong reactivity confined to the capsule, a weak extracellular staining in the hilus region, a weak staining of interstitial cells, but otherwise negative parenchymal cells.

Gastrointestinal tract, vascular and respiratory systems. The staining reactions for the two proteins were also remarkably similar both in intensity and localization in most of the tissues of the gastrointestinal tract, including the pancreas. Similar results were obtained in the lungs and the vascular system, including the heart. Respiratory epithelium and submucosa showed a weak and diffuse staining for fetuin, whereas α_2HS staining showed a pattern of strongly-reacting cells alternating with unstained cells in epithelium and submucosa (Fig. 4 A, B).

Thymus and liver. A major difference in the distribution and subcellular localization of the two antigens was observed in the thymus and liver. Lymphocytes were negative for both fetuin and α_2HS but the epithelial cells and the Hassall bodies derived from these cells were clearly positive for fetuin and negative for α_2HS (Fig. 6 A, B).

During early stages of development both fetuin-containing and α_2HS-containing hepatocytes were few in number and weak in staining intensity. In the late embryonic and early fetal period the number of positive cells and their staining intensity gradually increased. The positively-reacting hepatocytes exhibited a very different subcellular distribution of their reaction products. Thus fetuin staining resulted in a strong and diffuse reaction of the individual hepatocytes whereas α_2HS staining resulted in a distinct granular product in the perinuclear region (Fig. 7 A, B).

Discussion

The presence of fetuin in CSF and plasma of fetal sheep has been reported in previous publications (Dziegielewska 1982; Dziegielewska et al. 1980; Cavanagh et al. 1983). Quantitatively, it has been shown to be a major protein in CSF and plasma throughout a wide range of gestational age in this species.

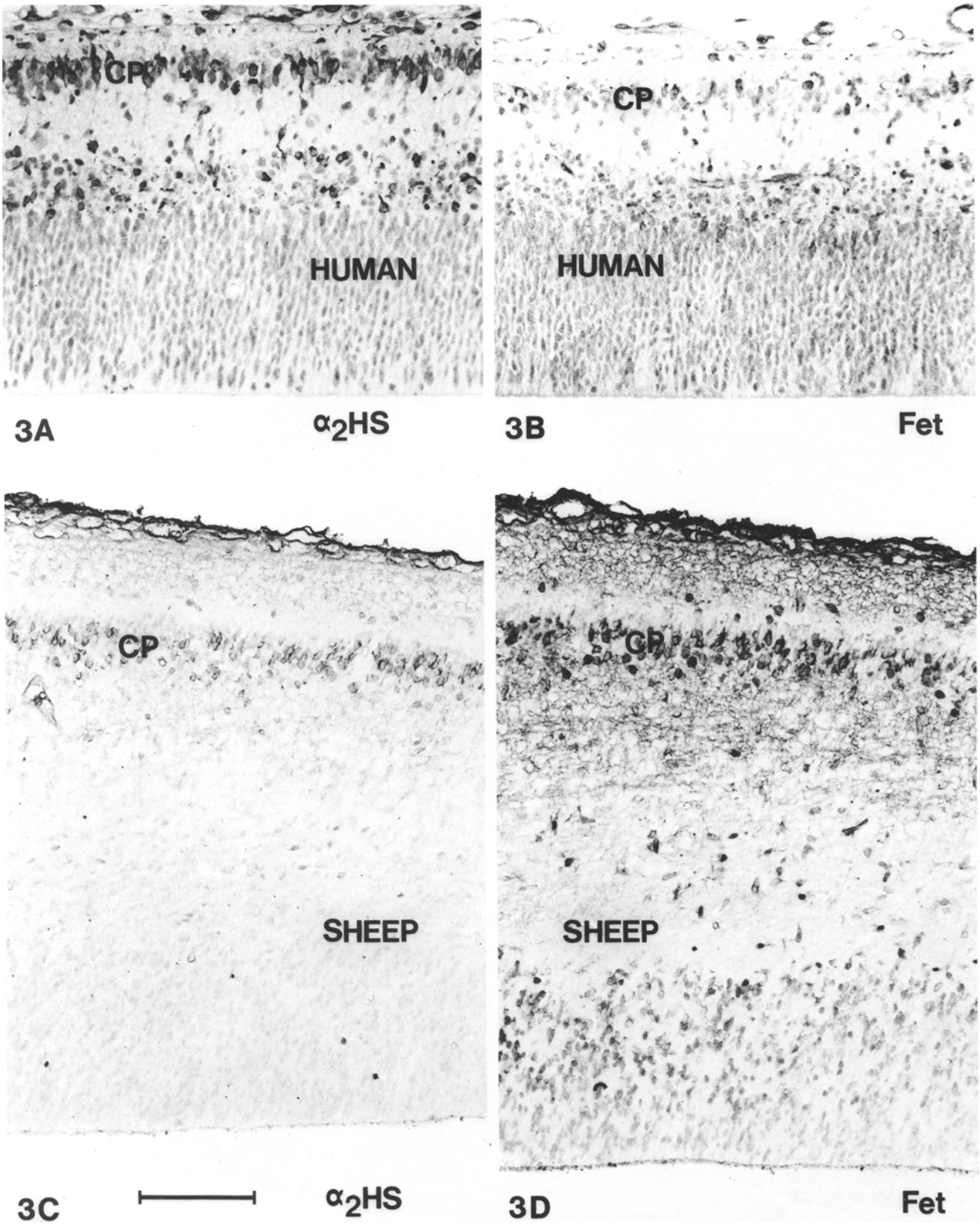
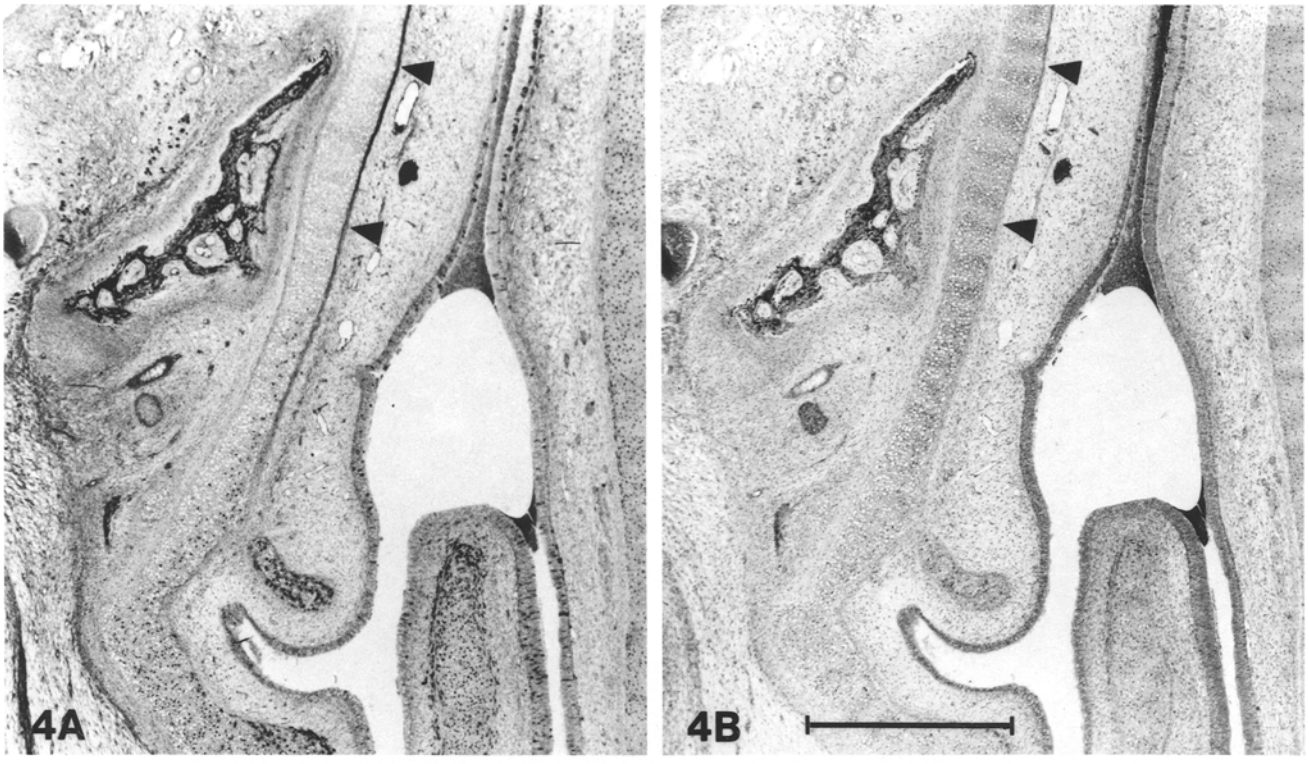
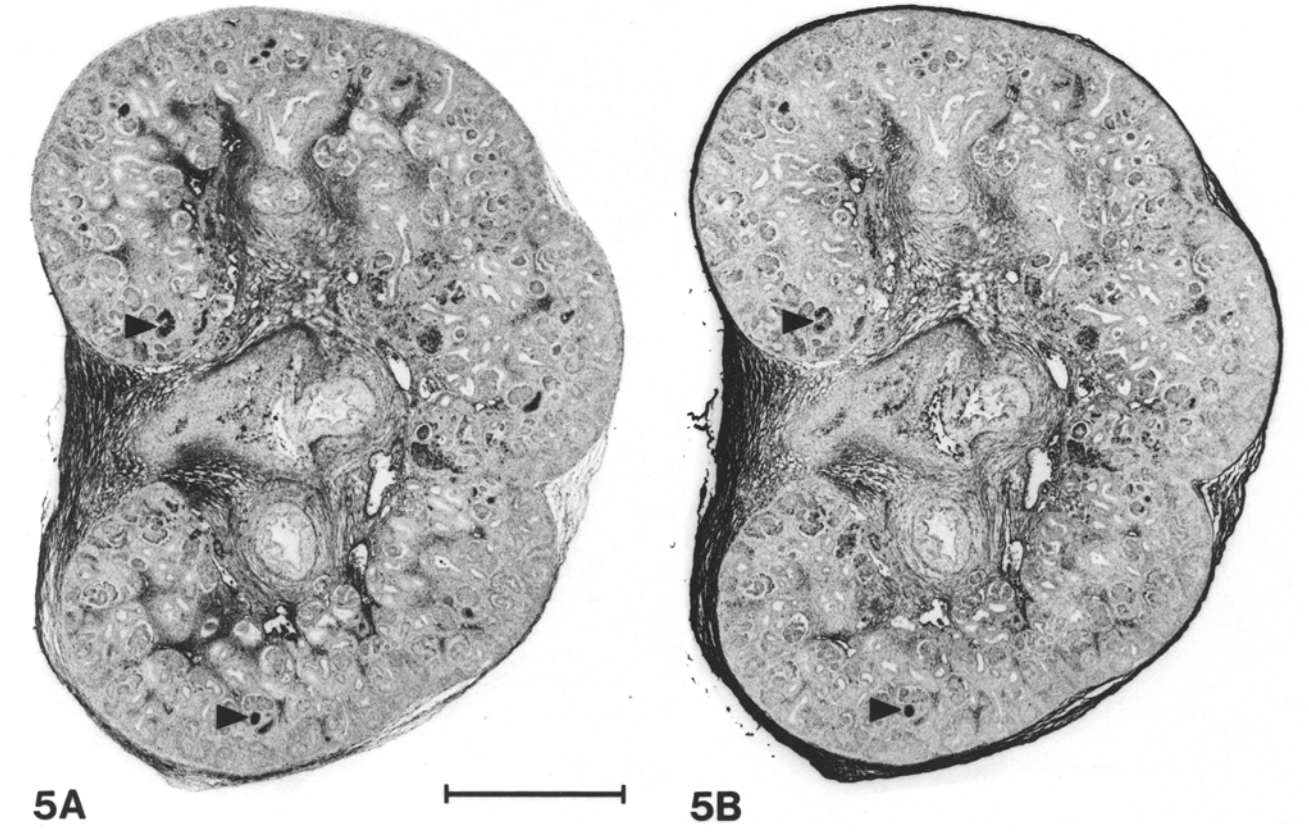
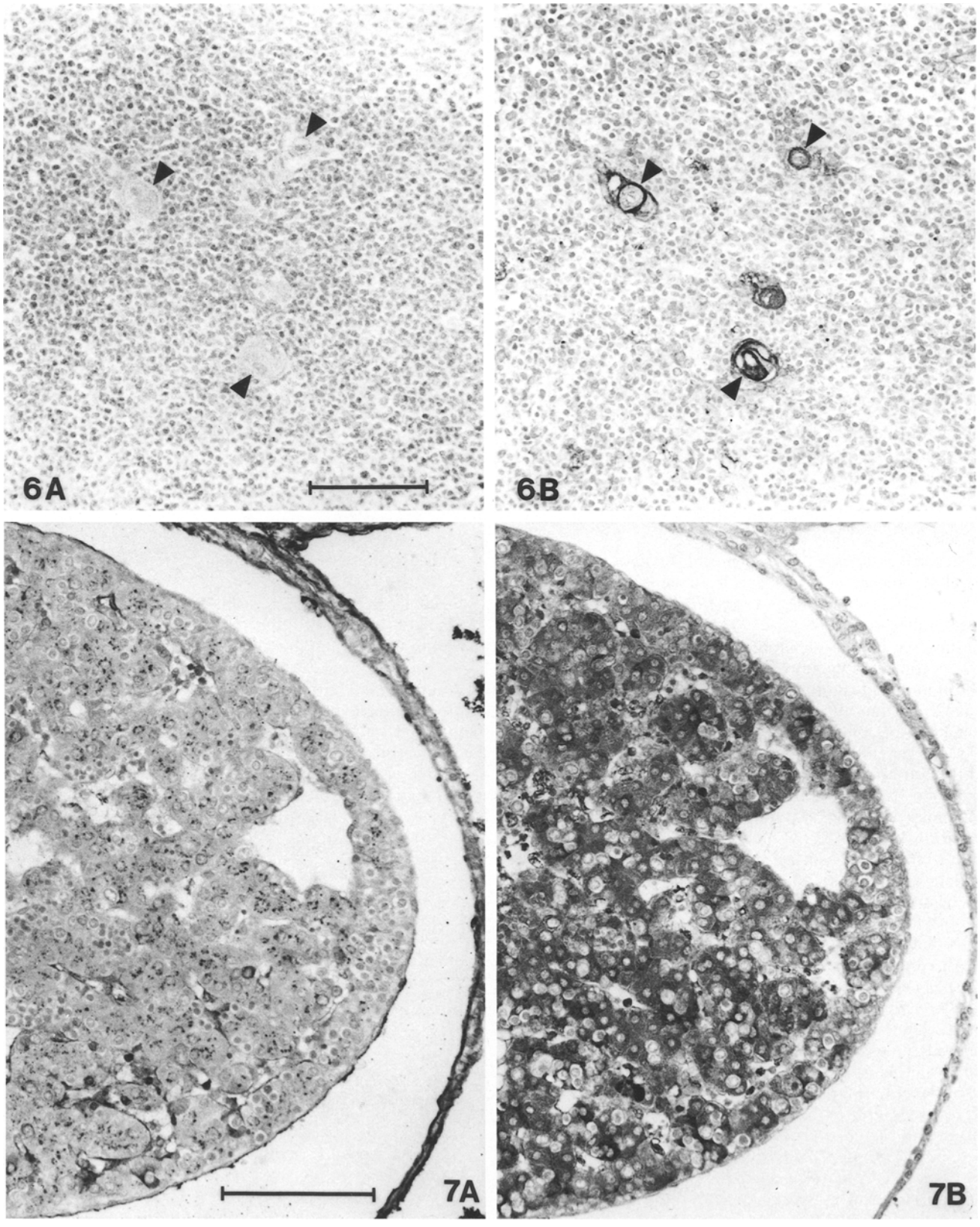


Fig. 3. Immunoperoxidase staining for α_2HS (**A**) and fetuin (**B**) of a 28 mm CRL human embryo. Note the many strongly α_2HS-stained cells in the cortical plate (*CP*) in **A** compared to the few weakly fetuin-stained cortical plate cells in **B**. The newly-formed cortical plate (*CP*) from a 36-day sheep embryo stained for α_2HS (**C**) and fetuin (**D**) demonstrates a reverse staining pattern. Bar indicates 100 μm





Figs. 4, 5. Immunoperoxidase staining for α_2HS (**4A** and **5A**) and fetuin (**4B** and **5B**). Consecutive sections of the anterior nose cavity (**4A, B**) from a 71 mm CRL human fetus and of a kidney (**5A, B**) from a 52 mm CRL fetus. Note the similarity of the staining reactions. The layer of osteoblasts (*arrowheads* in **4A**) reacts more strongly for α_2HS than for fetuin. In Fig. **5A, B** some intraluminal reaction products in some kidney tubules are indicated by *arrowheads*. Bars indicate 1 mm



Figs. 6, 7. Consecutive sections of thymus (**6A, B**) from a 140 mm CRL fetus and liver (**7A, B**) from a 71 m CRL fetus stained for α_2HS (**6A** and **7A**) and fetuin (**6B** and **7B**). The *arrowheads* in **6A, B** point to α_2HS-negative and fetuin-positive Hassall bodies. Note the granular α_2HS-staining reaction in **7A** compared with the strong, diffuse fetuin-staining reaction in **7B.** Bars indicate 100 μm

The immunochemical evidence presented in this paper (Fig. 1) shows that both α_2HS and fetuin are present in two widely different species: human and sheep. The immunocytochemical evidence confirms that this is the case and we have tried to take considerable care to distinguish between staining due to cross-reactivity and that due to specific staining for each antigen, particularly since both proteins appear to have a similar distribution in many organs. Absorption with the appropriate antigen has confirmed that the staining with each antiserum is specific. The blocking experiment has shown that, at least in the sheep cortical plate, both proteins are present in spite of cross-reactivity between anti-fetuin and α_2HS. Assessment of differences in staining intensity of the two proteins was difficult because of the use of heterologous and homologous antisera, quite apart from the usual problems met when comparing two antisera to different antigens. However, within a single section of a whole embryo stained for either fetuin or α_2HS it is possible to make some semi-quantitative deductions about the relative concentration of each antigen in different tissues. Thus it is clear, that staining for α_2HS in the human embryo is particularly strong in the cortical plate, but absent in the Hassall's corpuscles of the thymus. In contrast, fetuin staining in the cortical plate is weak but is stronger in the liver, gut, kidney and strongly positive in Hassall's corpuscles.

Extensive studies of the chemical structure (Alcaraz et al. 1981; Fisher et al. 1958; Gejyo and Schmid 1981; Marti et al. 1973; Matsuhima et al. 1982; Oshiro and Eylar 1968; Schultze and Heremans 1966) and possible biological functions (see below) of fetuin and α_2HS have been published. A considerable amount of work has also been done on the amino acid composition of these proteins. It appears to have gone unnoticed that they are remarkably similar. One method of comparing such data is the "Variation Index" (Metzger et al. 1968). Gejyo and Schmidt (1981) calculated the Variation Index for the "fast" and "slow" forms of α_2HS to be 2.1. Similar calculations have been made for human α_2HS and sheep fetuin using the data; this gives a Variation Index of 6.7. For comparison, human α-fetoprotein (data from Nishi et al. 1970) and sheep α-fetoprotein (data from Lai 1978), have a Variation Index of 11. Thus the difference in amino acid composition for the same protein (α-fetoprotein) from different species appears to be greater than the difference between α_2HS and fetuin for different species. The Variation Index for sheep and bovine fetuin (7.1) is scarcely different from that for α_2HS and fetuin (6.7). α_2HS consists of two polypeptide chains, one long (A chain) and one short (B chain). Both have been sequenced (Gejyo et al. 1983; Yoshioka et al. 1986). For fetuin, which is a single peptide chain, the first 40 amino acids of the N terminus have been sequenced (Alcaraz et al. 1981). The homology between the N terminal sequences of fetuin and of the A chain of α_2HS is about 60%. Thus fetuin and α_2HS are probably very closely related proteins which have diverged from a common gene; alternatively there may be a single gene controlling both proteins with post transcriptional differences at the mRNA level. The differences in carbohydrate chains are probably a reflection of differences in subcellular processing. This possibility is supported by the observation that the immunocytochemical staining for the two proteins is different in some organs. For example, in the liver, α_2HS staining has a granular appearance which may be associated with the Golgi apparatus whereas fetuin staining is diffuse, and is perhaps within the endoplasmic reticulum. Work is now in progress to investigate these possibilities.

A wide range of functions has been ascribed to fetuin itself or to a serum fraction containing fetuin. Most effects were described in culture of various tissues. Thus fetuin was observed to stimulate adherence and growth of cells (Fisher and Puck 1958; Puck et al. 1968), sustain (together with albumin) growth in culture (Rizzino and Shermann 1979), and be responsible for spreading of cells (Knox et al. 1979). Recently, it was shown (Raff et al. 1983) that some preparations of fetuin cause differentiation of type-2 astrocytes in cultures of postnatal rat optic nerve.

Other possible functions of fetuin also suggested were: binding of thyroxin (Fisher and Lam 1974), trypsin inhibitor activities (Galembeck and Cann 1974), inhibition of viral and lectin haemaglutination (Yachnin 1975) and lymphocyte stimulating properties (Hsu and Floyd 1976).

On the other hand, possible functions of α_2HS have mainly been investigated in relation to the finding that it is present in bone-forming tissues (Dickson et al. 1975) and that it has a high affinity for Ba^+ and Ca^{++} (Triffitt et al. 1976). The plasma concentration of α_2HS is reduced in some acute disorders (negative acute phase protein, e.g., Baskies et al. 1980) in malnutrition (Schelp et al. 1980) and in Paget's disease of bone (Ashton and Smith 1980). Other functions proposed for α_2HS include DNA-binding (Lewis and André 1978) opsonic properties (van Oss et al. 1974) and enhancement of phagocytosis (Lewis and André 1981). The lack of similarity in these proposed functions for α_2HS and fetuin may simply be a reflection of the fact that there has been no previous attempt to compare them. The striking distribution of fetuin in Artiodactyla, such as sheep and α_2HS in human in the very early cortical plate suggests that these closely related glycoproteins may be of some special significance in the development of the cortical plate and initial connections with other brain regions. The widespread distribution of these glycoproteins in other developing tissues suggests that they may also play a more general role.

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References

Alcaraz G, Marti J, Moinier D, Fougereau M (1981) NH_2-terminal sequence of calf fetuin. Biochem Biophys Res Comm 99:30–36

Ashton BA, Smith R (1980) Plasma α_2HS-glycoprotein concentration in Paget's disease of bone: its possible significance. Clin Sci 58:435–438

Baskies AM, Chretien PB, Weiss JF, Makuch RW, Beveridge RA, Catalona WJ, Spiegel HE (1980) Serum glycoproteins in cancer patients: First report of correlations with in vitro and in vivo parameters of cellular immunity. Cancer 45:3050–3060

Boulder Committee (1970) Embryonic vertebrate central nervous system; Revised terminology. Anat Rec 166:257–261

Cavanagh ME, Cornelis ME, Dziegielewska KM, Luft AJ, Lai PCW, Lorscheider FL, Saunders NR (1982) Proteins in cerebrospinal fluid and plasma of fetal pigs during development. Dev Neurosci 5:492–502

Cavanagh MR, Cornelis MEP, Dziegielewska KM, Evans CAN, Lorscheider FL, Møllgård K, Reynolds ML, Saunders NR (1983) Comparison of proteins in CSF of lateral and IVth ventricles during early development of fetal sheep. Dev Brain Res 11:159–167

Cavanagh ME, Møllgård K (1985) An immunocytochemical study of the distribution of some plasma proteins within the developing forebrain of the pig with special reference to the neocortex. Dev Brain Res 17:183–194

Dickson IR, Poole AR, Veis A (1975) Localisation of plasma α_2HS glycoprotein in mineralising human bone. Nature 256:430–432

Dziegielewska KM, Evans CAN, Fossan G, Lorscheider FL, Malinowska DH, Møllgård K, Reynolds ML, Saunders NR, Wilkinson S (1980) Proteins of CSF and plasma of fetal sheep during development. J Physiol 300:441–445

Dziegielewska KM, Evans CAN, Malinowska DH, Møllgård K, Reynolds ML, Saunders NR (1981) Plasma proteins in fetal sheep brain: blood-brain barrier and intracerebral distribution. J Physiol 318:239–250

Dziegielewska KM, Saunders NR (1982) Transferrin in fetal sheep cerebrospinal fluid and plasma during gestation. Comp Biochem Physiol 73A:327–329

Dziegielewska KM, Bock E, Cornelis MEP, Møllgård K, New H, Saunders NR (1983) Identification of fetuin in human and rat fetuses and in other species. Comp Biochem Physiol 76A:241–245

Felgenhauer K (1974) Protein size and cerebrospinal fluid composition. Klin Wochenschr 52:1158–1164

Fisher DA, Puck TT, Sato G (1958) Molecular growth requirements of single mammalian cells: the action of fetuin in promoting cells attachment to glass. Proc Natl Acad Sci 44:4–10

Fisher DA, Lam RW (1974) Thyroid hormone binding by bovine and ovine fetuin. Endocrinology 94:49–54

Galembeck F, Cann JR (1974) Fetuin as a trypsin inhibitor. Arch Biochem Biophys 164:326–331

Gejyo F, Schmid K (1981) Purification and characterization of the two forms of human plasma α_2HS-glycoprotein. Biochem Biophys Acta 671:78–84

Gejyo F, Chang J-L, Burgi W, Schmid K, Offner GD, Troxler RG (1983) Characterization of the B-chain of human plasma α_2HS-glycoprotein. J Biol Chem 258:4966–4971

Hsu CCS, Floyd M (1976) Lymphocyte stimulating and immunochemical properties of fetuin preparations. Prot Biol Fluids 24:295–20

Knox P, Griffiths S, Whately JG (1979) Fetal calf serum and the adhesion of cells in culture. Biochem Soc Trans 7:1011–1012

Köhler G, Milstein C (1975) Continuous cultures of fused cells secreting antibodies of predefined specificity. Nature 256:495–497

Lai PCW (1978) Alpha-fetoprotein: chemical characterization and fetal-maternal distribution in the rat and sheep PhD Thesis, Calgary, Alberta.

Lewis JG, André CM (1978) A serum DNA-binding protein absent in malignant disease. FEBS Lett 92:211–213

Lewis JG, André CM (1981) Enhancement of human monocyte phagocytic function by α_2HS-glycoprotein. Immunology 42:481–487

Macini G, Carbonara AO, Heremans JF (1965) Immunochemical quantification of antigens by single radial immunodiffusion. Int J Immunochem 2:235–254

Marin-Padilla M (1978) Dual origin of the mammalian neocortex and evolution of the cortical plate. Anat Embryol 152:109–126

Marti J, Aliau S, Bonfils C, Vigne C, Moretti J (1973) Preparation, constantes physiques et constitution chimique de la fetuine d'agneau. Biochim Biophys Acta 303:348–359

Matsuhima K, Cheng M, Migita S (1982) Purification and physicochemical characterization of human α_2HS-glycoprotein. Biochim Biophys Acta 701:200–205

Metzger H, Shapiro MB, Mosimann JE, Vinton JE (1968) Assessment of compositional relatedness between proteins. Nature 291:1166–1168

Møllgård K, Jacobsen M (1984) Immunohistochemical identification of some plasma proteins in human embryonic and fetal forebrain with particular reference to the development of the neocortex. Dev Brain Res 13:49–63

Møllgård K, Reynolds ML, Jacobsen M, Dziegielewska KM, Saunders NR (1984) Differential immunocytochemical staining for fetuin and transferrin in the developing cortical plate. J Neurocytol 13:497–502

Nishi S (1970) Isolation and characterisation of human fetal α-globulin from the sera of fetuses and a hepatoma patient. Cancer Res 30:2507–2513

Oshiro Y, Eylar EH (1968) Physical and chemical studies on glycoproteins. III. The microheterogeneity of fetuin, a fetal calf serum glycoprotein. Arch Biochem Biophys 127:476–489

van Oss CJ, Gillman CF, Bronson PM, Border JR (1974) Opsonic properties of human serum alpha-$_2$HS glycoprotein. Immunol Comm 3:329–335

Pedersen KO (1944) Fetuin, a new globulin isolated from serum. Nature 154:575

Puck TT, Waldren A, Jones C (1968) Mammalian cell growth proteins. 1. Growth stimulation by fetuin. Proc Natl Acad Sci 59:192–199

Putnam WF (1984) The plasma proteins, Vol IV. Academic Press, New York

Raff MC, Miller RH, Noble M (1983) A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on culture medium. Nature 303:390–396

Rizzino A, Sherman MI (1979) Development and differentiation of mouse blastocysts in serum-free medium. Exp Cell Res 121:221–233

Reynolds ML, Møllgård K, Saunders NR (1983) The distribution of plasma proteins during early embryonic development in the sheep. Anat Embryol 168:227–240

Reynolds ML, Møllgård K (1985) The distribution of plasma proteins in the neocortex and early allocortex of the developing sheep brain. Anat Embryol 171:41–60

Reynolds ML, Sarantis MEP, Lorscheider FL, Saunders NR (1986) Fetuin as a measurer of cortical plate cells in the fetal low neocortex: a comparison of the distribution of fetuin, α_2HS-glycoprotein, α-fetaprotein and albumin during early development. Anat Embryol 175 (in press)

Schelp FP, Thanangkul O, Supawan V, Pongpaew P (1980) α_2HS-glycoprotein serum levels in protein-energy malnutrition. Br J Nutr 43:381–383

Schuller E, Allinquant B, Garcia M, Lefevre M, Moreno P, Tampe L (1971) Electro-immunodiffusion des proteines du liquide cephalo-rachidien. Clin Chim Acta 33:5–11

Schultze HE, Heremans JF (1966) Molecular biology of human proteins with special reference to plasma proteins. Vol 1. Nature and metabolism of extracellular proteins. Elsevier, Amsterdam

Spiro MJ, Spiro RG (1962) Composition of the peptide portion of fetuin. J Biol Chem 237:1507–1510

Triffitt JT, Gebauer U, Ashton BA, Owenn ME, Reynolds JJ (1976) Origin of plasma α_2HS-glycoprotein and its accumulation in bone. Nature 41:226–227

Weeke B (1973) Rocket Immunoelectrophoresis. In: Axelsen NH, Kroll J, Weeke B (eds) A Manual of Quantiative Electrophoresis. Universitetsforlaget, Oslo, pp 15–35

Yachnin S (1975) Fetuin, an inhibitor of lymphocyte transformation. The interaction of fetuin with phytomitogens and a possible role for fetuin in fetal development. J Exp Med 141:242–256

Yoshioka Y, Gejyo F, Marti T, Rickli EE, Bürgi W, Offner G, Troxler RF, Schmid K (1986) The complete amino acid sequence of the A-chain of human plasma of α_2HS-glycoprotein. J Biol Chem 261:1665–1676

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