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Sites of prealbumin production in the human fetus using the indirect immunoperoxidase technique

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Summary. An indirect immunoperoxidase technique was used to investigate the site of prealbumin synthesis in the human fetus from between 8 and 43 weeks. Prealbumin staining was noted in the A cells of the fetal pancreas, both in the primary and secondary generations of the islets of Langerhans, from as early as 12 weeks gestation. Electron microscopy localized the staining to the cytoplasmic secretory granules. Prealbumin was also present in the gastrointestinal mucosa in cells which had a distribution similar to that of argentaffin cells. Positive staining was noted in the lining epithelium of the renal proximal convoluted tubules, but ultrastructural studies indicated that this was probably related to reabsorption, and not to synthesis.

Key words: Human prealbumin – Fetus – APUD cells

Introduction

Prealbumin, also known as thyroxine binding prealbumin or vitamin A-transporting protein, is a plasma protein with a molecular weight of 54,980 (Kanda et al. 1974). As its synonyms suggest it has an unusual dual transport role involving thyroxine and vitamin A. It is present in human fetal cord blood from as early as 11.5 weeks gestation (Rossi et al. 1970), and is a major plasma protein in the adult (Ritchie 1979). The serum level in the adult is within the range 18.7–35.0 mg/100 ml (Hutchinson et al. 1981) but the levels decrease in association with several pathological conditions, including surgical trauma (Keyser 1979), burn injuries (Moody 1982), acute infection (Oppenheimer 1968), hyperthyroidism (Smith and Goodman 1971), cystic fibrosis (Smith et al. 1972), liver disease (Agostini et al. 1968;

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Hutchinson et al. 1981) and in protein-calorie malnutrition (Ingenbleck et al. 1972, 1975).

Work by Gitlin and Baisucci (1969), using tissue culture techniques from human fetuses provided evidence to suggest that the liver cells are the site of synthesis of prealbumin. However, more recent work using an indirect immunoperoxidase technique on adult tissue failed to demonstrate prealbumin in the liver and instead it was found to be present in the pancreatic islets of Langerhans (Jacobsson et al. 1979). In view of these conflicting reports, the distribution and sites of synthesis of prealbumin in human fetal tissue was studied, using an indirect immunoperoxidase technique.

Materials and methods

The tissues studied were liver, pancreas, pyloric antrum, body of stomach, small intestine, colon, kidney, spleen, thymus, lung, brain and placenta. The tissue was collected from up to 25 human fetuses sent to the Department of Pathology, Aberdeen University Medical School, Aberdeen, for routine autopsy examination. Their gestational ages ranged from 8 to 43 weeks (mean 21.5 weeks). Fetal age was calculated both by considering the menstrual age and also by measuring the crown-rump and footlengths which were then compared to the standard tables of fetal measurement (Streeter 1920). Tissue from any fetus showing maceration, autolysis, intrauterine infection, or maldevelopment, was excluded from the study. A section from each tissue block was also stained by haematoxylin and eosin to further confirm a normal histological appearance. The interval between delivery of the fetus and fixation of the tissue varied from less than 5 min to 48 h. In those fetuses of less than 28 weeks gestation this time interval was usually less than 3 h. All fetuses were stored at 4° C immediately on delivery until tissue examination and fixation were possible. The tissues selected were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned to 5 μm and mounted on polylysine coated microscope slides to prevent the sections washing off during the long staining procedure. All sections were stained for human prealbumin using the indirect immunoperoxidase technique (Gallon et al. 1981). The antisera used were normal swine serum (Dakopatts A/S, Copenhagen, Denmark) rabbit immunoglobulins to human prealbumin (Dakopatts A/S) swine antiserum against rabbit immunoglobulins (Dakopatts A/S), and peroxidase-anti-peroxidase complex (Dakopatts A/S and Scottish Antibody Production Unit, Carluke, U.K.).

A negative control of each tissue section underwent the same procedure as above using normal rabbit serum (Scottish Antibody Production Unit) in place of the rabbit antiserum to human prealbumin. In addition the specificity of the prealbumin antiserum has been shown by absorption studies using purified prealbumin (Liddle et al. 1984). Each batch of sections stained also included a section of fetal pancreas to act as a positive control.

In order to compare the site of prealbumin synthesis with that of glucagon synthesis, serial sections were cut from the pancreas of three fetuses of 14, 22 and 40 weeks gestation. Paired sections were then stained by the indirect immunoperoxidase technique using rabbit antisera to prealbumin, and rabbit antisera to glucagon (Guildhay Ltd., Guildford, U.K.). Photomircrographs were taken of each member of the pair and the cells staining for prealbumin and for glucagon compared.

Ultrastructural studies of pancreas and kidney were carried out to determine the distribution of prealbumin within the cell (Hashida 1980; Bancroft 1982).

Paraffin sections stained by the indirect immunoperoxidase technique as described above, omitting counterstaining, were rinsed in phosphate buffer prior to 15 min post fixation in 1% osmium tetroxide, then dehydrated though graded alcohol and placed in propylene oxide before overnight impregnation with a 50:50 propylene oxide-Epon mixture. After the sections were embedded in Epon-araldite in vacuums (5 h at 40° C and 24 h at 60° C with fresh Epon) they were warmed on a hotplate and a selected area containing positively stained cells prised off, attached to a beam capsule block of Epon-araldite and sectioned. Ultrathin sections were viewed using a Jeol 100-S transmission electron microscope.

Table 1. Tissues staining positively for prealbumin

Tissue	Number of fetuses	Mean gestation age (weeks)	Age range (weeks)	Percentage positive in fetuses >11 weeks
Liver	10	20.4	8–36	0
Pancreas	25	21.8	8–43	100
Stomach pylorus	8	20.4	8–36	100
Stomach body	8	20.0	8–36	100
Small intestine	13	20.1	8–38	100
Colon	9	24.0	8–38	100
Kidney	8	17.6	8–24	100
Spleen	7	21.7	8–40	0
Thymus	6	27.2	17–40	0
Lung	7	24.7	8–40	0
Brain	5	19.6	16–24	0
Placenta	5	21.2	16–28	0

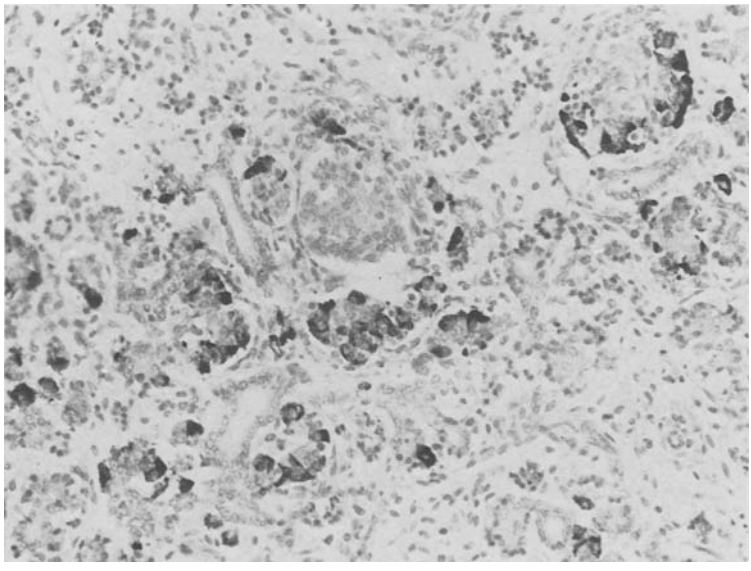

Fig. 1. Prealbumin positive cells in fetal pancreas, age 14 weeks. Positive cells are at periphery of islets and in paratubular areas. PAP ×90

Results

Our results are presented in Table 1. Positive staining was present in pancreas, stomach, small intestine, colon and kidney. Prealbumin was not detected in any sections of liver, spleen, thymus, lung, brain or placenta. Of the 25 fetal pancreas (gestation age range 8–36 weeks) studied, strong positive staining was noted in the cytoplasm of selected cells from all fetuses of 12 weeks gestation or more.No staining was detected in the pancreas of the 8 week embryo. At 12 weeks gestation cells staining positively for prealbumin composed a substantial population of the developing islet tissue,

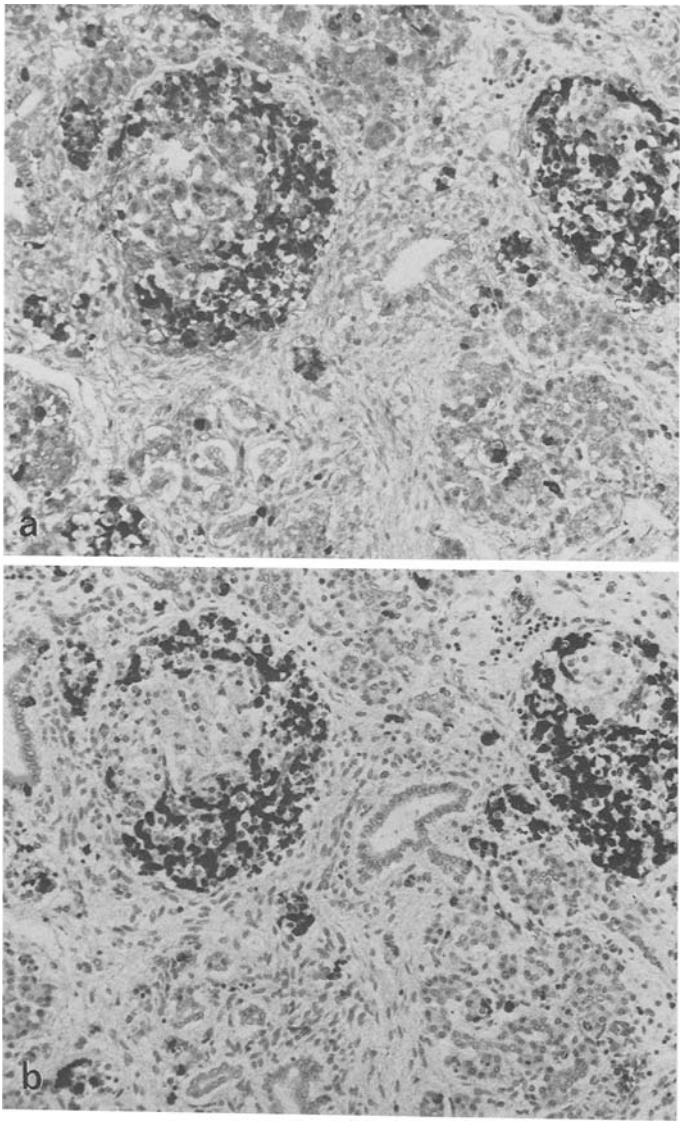

Fig. 2. **a** Prealbumin positive cells in fetal pancreas, age 14 weeks. Distribution matches that of glucagon positive cells in serial section in **b**. ×150. **b** Staining for glucagon in serial section of **a** shows the distribution to be similar. PAP ×150

being located in the periphery of the islet. Positive cells were also located either singly or in small groups between the ductules. At 14 weeks to 43 weeks positive cells were identified not only in the periphery of the islets and in the paraductular regions, but also in small clusters and singly within the rapidly developing exocrine tissue (Fig. 1).

Comparison of paired serial sections of pancreas (at selected gestational ages of 14, 22 and 40 weeks) stained for prealbumin, and for glucagon,

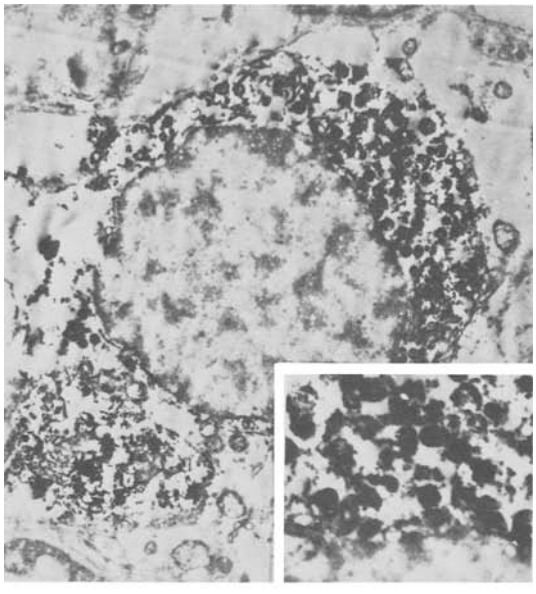

Fig. 3. Prealbumin positive cytoplasmic granules in A cells of fetal pancreas, age 18 weeks. Diameter of granules 280 nm. Paraffin embedded autopsy material. PAP ×5,000. *Inset* ×10,000

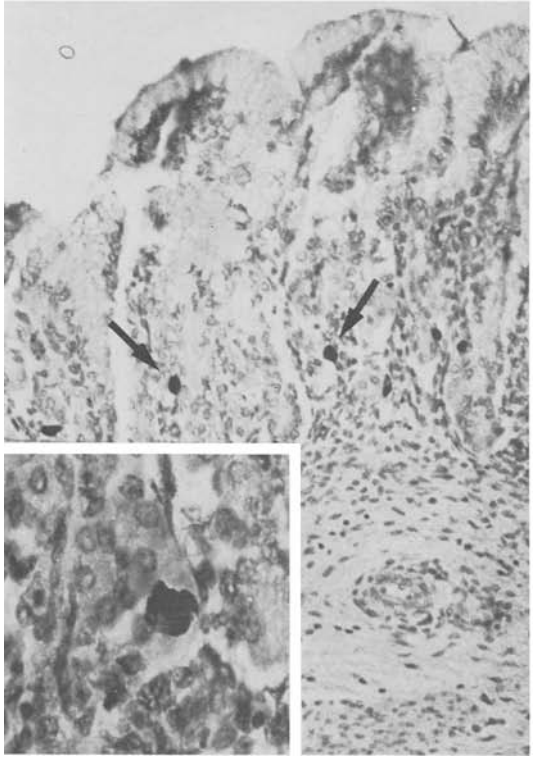

Fig. 4. Prealbumin positive cells (*arrows*) at base of fetal gastric glands, age 34 weeks. PAP ×160. *Inset* ×400

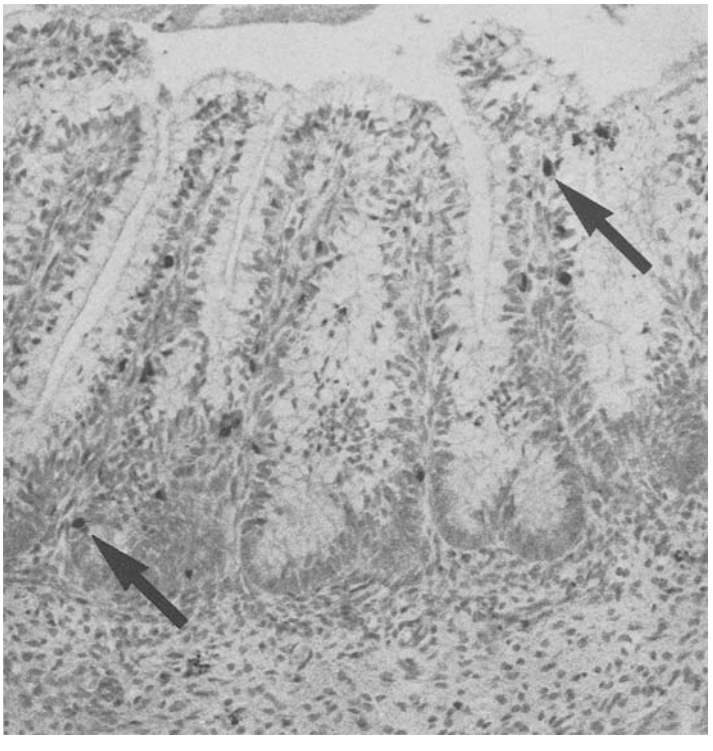

Fig. 5. Prealbumin positive cells (*arrows*) at various levels in crypts, fetal colonic mucosa, age 18 weeks. PAP ×180

showed the distribution of prealbumin to match that of glucagon, both within the islets, and in the extra islet cells (Fig. 2a, b).

Ultrastructural examination revealed strong positive staining within the cytoplasmic granules of the A cells, and also more diffusely throughout the cytoplasm (Fig. 3). These granules were in appearance typical of endocrine secretory granules, and had a diameter of the order of 280 nm.

In sections taken from the fetal gastrointestinal tract it was noted that tissue preservation was poor in some cases, with loss or disruption of the epithelium to varying degrees. However, in stomach (both body and pylorus), small intestine and colon, positive staining was found in small single cells in the intact epithelial tissue. These cells, which were lying on the basement membrane, in the stomach were localised at the base of the gastric glands (Fig. 4), and in the small intestine and colon at various levels within the crypts (Fig. 5). In the 8 week old embryo, no positive cells were found, but positive cells were found at these sites in the intestinal tract of all the fetuses from 12 weeks gestation onwards.

In renal tissue positive staining for prealbumin was present in the epithelium of the proximal convoluted tubules of all kidneys from 16 weeks gestation to term. The staining density of the tubular cytoplasm increased markedly with increasing maturity. At 16 weeks only small numbers of inclusion

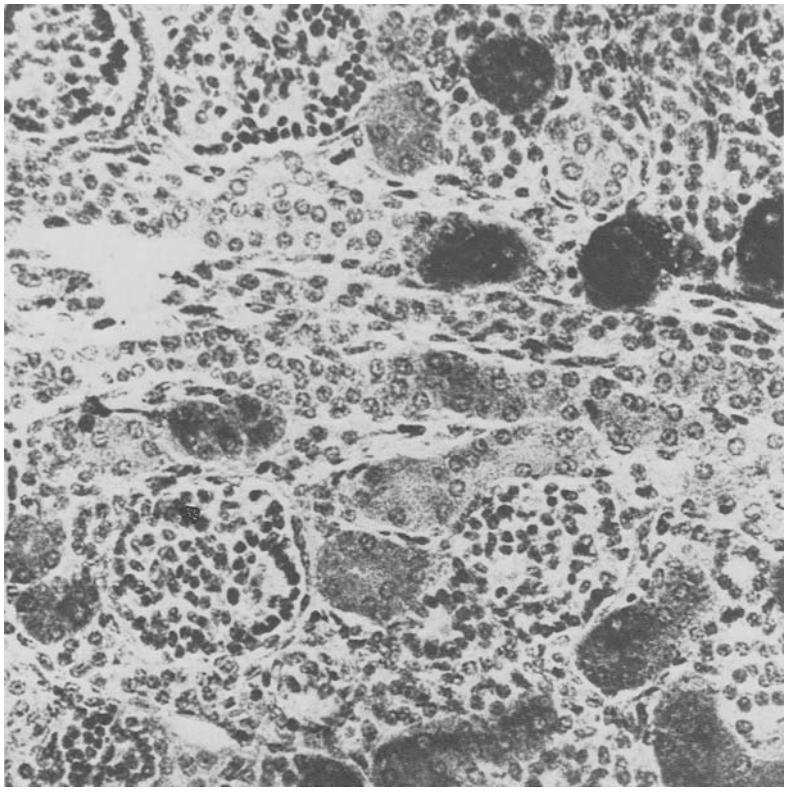

Fig. 6. Proximal tubules of fetal kidney show positive staining for prealbumin, age 24 weeks. PAP $\times 200$

bodies, scattered throughout the cytoplasm, showed positivity, whilst at 24 weeks the entire cytoplasm was heavily stained. Variation in staining intensity was noted amongst tubules from the same tissue section, though the intensity was uniform amongst cells from a similar level of the tubule (Fig. 6). Ultrastructural examination showed positive staining of large polymorphic vacuoles which appeared to be formed from the fusion of smaller ones and their staining was particulate and peripheral. They had the appearance of pinocytotic vacuoles.

No positive staining for prealbumin was found in the liver, spleen, lung, brain, thymus or placenta at any of the gestational ages investigated, although in all cases positive control sections were staining strongly.

Discussion

The role of the liver as the major site of synthesis for many plasma transport proteins, albumin being a prime example, has been accepted for many years. Evidence from fetal tissue culture studies (van Furth et al. 1965; Gitlin and Biasucci 1969) indicated that the liver is also the site of prealbumin

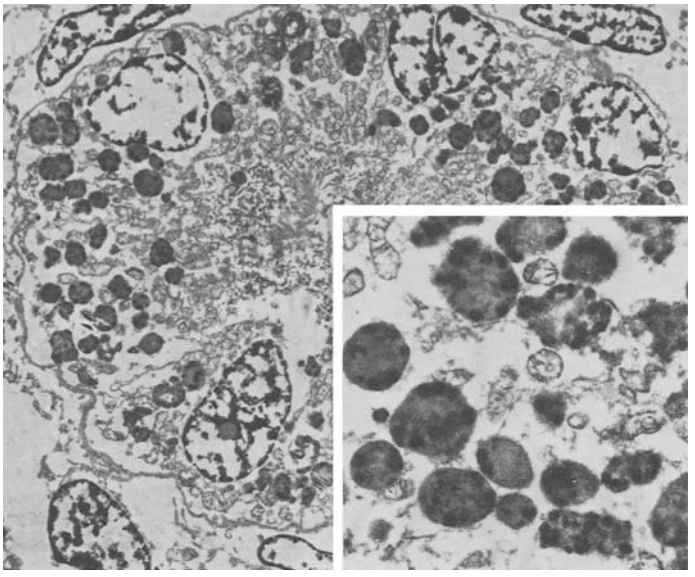

Fig. 7. Prealbumin positive, polymorphic vacuoles in cytoplasm of lining cells of proximal renal tubules, age 18 weeks. PAP ×2,500. *Inset* ×7,500

synthesis in man, and the indirect evidence of decreased prealbumin levels in conditions of liver disease appears to support this (Hallen and Laurell 1972; Hutchinson et al. 1981). However, the results of this study clearly show that prealbumin is not present in hepatocytes using an indirect immunoperoxidase technique. Recent studies on human adult tissue (Liddle et al. 1984; Jacobsson et al. 1979), using the same technique have also failed to detect prealbumin in the liver.

The use of freshly fixed sections of fetal liver in this study, and of surgical biopsies from adult liver by Liddle et al. (1984), preclude the possibility of prealbumin degradation occurring to any substantial degree and presenting as a falsely negative result. It could be argued that because of the large size of the liver only very low levels of production at the individual cellular level may occur, but this is unlikely owing to the high sensitivity of the technique and knowing the strong intensity of the staining in positive cells of other tissues. For these reasons we believe that the fetal liver does not produce prealbumin.

The results from fetal pancreas show clearly that prealbumin is detectable in the same cells of the islets of Langerhans as glucagon. This complements the findings of Liddle et al. (1984) who demonstrated that prealbumin was present in the glucagon producing A cells in the adult.

Positive staining cells followed the same patterns of A cell development reported by Liu and Potter (1962). They describe two generations of islet cell development, the primary generation originating from paratubular cell buds. These cells detach themselves from the ducts and establish primary islets, comprising of a peripheral band of A and D cells surrounding a

core of B cells. Primary islets continue to enlarge and increase in number until 20 weeks gestation when they begin to disintegrate. In contrast, the cells of the second generation of islets arise from amongst the epithelial cells of terminal ducts at around 16 weeks gestation. Throughout intrauterine life the secondary islets formed increase in number and size, going on to form the characteristic islets of the adult pancreas. Prealbumin staining is present at an early stage in the development of both generations and there would appear to be no difference between the staining of early single cells, nor between primary and secondary islet A cells.

In the adult, A cell secretory granules range in diameter from 200 to 400 nm which corresponds to the size of the positive staining cytoplasmic granules in the fetus. Liddle et al. (1984) also noted prealbumin staining granules to be of similar size to those staining for glucagon. Positive staining in the cytoplasm indicates the presence of prealbumin in association with disrupted rough endoplasmic reticulum. Both these factors then lend strong support to A cell origin of prealbumin, along with glucagon, with its storage in the cytoplasmic granules before exocytosis into adjacent islet capillaries.

Pearse and Polak (1981) proposed that the diffuse family of polypeptide secreting cells of the gastrointestinal tract and pancreatic islets had a common embryological origin, though recently the neural crest origin has been questioned (Stevens and Moore 1983). These cells have various biochemical and ultrastructural similarities and were classed as APUD cells (amino precursor uptake and decarboxylation) by Track (1980).This link between the glucagon producing A cells of the pancreas and the enterochromaffin cells of intestine, some of which secrete enteroglugacon, makes it less suprising to find prealbumin positive cells in the intestine. The positive cells are small, occurring singly and lie against the basement membrane, which fits with the description of the enterochromaffin cells.

A similar APUD-like distribution of prealbumin positive cells was reported by Liddle et al. (1984) for adult gut. However, the proportion of tissues with positive staining was less than that found in this study. This difference is probably due to the comparatively large areas of intestine surveyed in each fetal tissue block scaled in proportion to the adult blocks, making detection of positive cells more likely.

The staining in the kidney is localised to large polymorphic vacuoles within the proximal convoluted tubules. Such bodies have been described both in mouse and human fetal kidney and are thought to represent protein which passes through the temporarilty abnormally permeable glomeruli and is then reabsorbed in the proximal tubule cell (Davies 1954; Clark 1957). In contrast with the positive A cells, cytoplasmic membranes were unstained thus discounting the possibility of prealbumin synthesis within the tubule cells. This is also supported by the fact that tubular staining increased with increasing age as would be expected with a rising serum level during gestation (Rossi et al. 1970; Stabilini et al. 1968).

Krzalic (1982) suggested that a local increase in production may explain the rise in prealbumin CSF levels in patients suffering from multiple sclerosis. Although the brain tissues investigated both in this project and by Liddle

et al. (1984) show no evidence of prealbumin staining it would be necessary to look for it in the arachnoid plexus to confirm or deny this hypothesis.

The negative findings in the other fetal tissues investigated are in direct agreement with the adult studies of Liddle et al. (1984). Why, therefore, liver disease should be associated with a lowering of serum prealbumin is still not clear. It is well known that alcohol, a major cause of adult liver disease, is an important pathogenic agent in chronic pancreatitis, adversely affecting both exocrine and endocrine functions. In addition, we have observed that infants with neonatal hepatitis or cirrhosis, frequently have hyperplasia of the endocrine tissue of the pancreas. This association has also been recorded by other authors (Patton et al. 1972; Greco and Finegold 1973) but no explanation has been offered. It appears that panceatic endocrine function, and human prealbumin production, may be regulated by the liver in some way and that this is the explanation for the abnormally low levels of human prealbumin found in patients with liver disease.

In conclusion, our search for the sites of prealbumin synthesis in the human fetus demonstrated its occurrence in the APUD cells of the fetus, namely in the A cells of the islets of Langerhans and in the enterochromaffin cells of the intestine. The repeated failure do demonstrate prealbumin in the liver at all ages is in agreement with the earlier studies on adult tissues. This conflicts with the current belief that prealbumin is synthesised in the liver and suggests that the actual site for prealbumin synthesis is the A cells of the pancreas.

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