Ontogeny of tissue specific protein markers provides useful experimental model for investigating regulation of gene expression during differentiation. Alpha fetoprotein and albumin are important specific differentiation markers and they bear a reciprocal relationship during ontogeny. The following lines summarize the results obtained regarding ontogeny of AFP and albumin in different tissues of rat as well as developmental profile of AFP in human brain.

AN IMPROVED PROCEDURE FOR ISOLATION OF AFP

A direct negative immunoabsorption procedure was developed to isolate homogeneous preparation of rat and human AFP from amniotic fluid. It requires no more than two steps: (a) removal of adult serum proteins present in amniotic fluid by a direct negative immunoabsorption (immunoprecipitation) with rabbit anti-normal adult rat or human serum IgG, and (b) removal of excess of immunoglobulins using DEAE-cellulose chromatography. Purified AFP was characterized by double immunodiffusion, immunoelectrophoresis, crossed immunoelectrophoresis, PAGE, SDS-PAGE, Con-A affinity chromatography, and estradiol binding. Rat AFP has $K_a = 1.4 \times 10^8 \text{ M}^{-1}$ for estradiol-17β with one binding site for estradiol per mole of AFP.

Isolation of homogeneous AFP by a direct negative immunoabsorption is a significant improvement over the time consuming, multistep procedures reported for AFP. In
addition, the present procedure did not require specific antiserum against AFP and employed very gentle isolating conditions. The procedure yielded 87% recovery of AFP.

ONTogeny and distribution of AFP in Feto-neonatal tissues of Rat and Human Fetal Brain

Most of the feto-neonatal tissues, except thymus, spleen and pancreas, of rat were found to contain elevated levels of AFP. High levels of AFP observed during fetal life in various tissues registered a decline with development. Ontogeny of AFP in these tissues was different from that observed in serum. AFP disappears after 7 d of postnatal life (P) in brain and adrenals; 14 P in lung, heart, stomach, intestinal and kidney; 21 P in liver and skin. Determination of heme concentration in these tissue extract revealed that contamination of AFP from fetal blood could not account for more than 5-10% of the observed levels of AFP in various feto-neonatal tissues of rat.

AFP present in various feto-neonatal tissues of rat was immunologically identical to serum AFP. AFP from serum, brain, skin and kidney also displayed a similar electrophoretic mobility on 10% PAGE and IEP. Estradiol binding assay of AFP in brain and skin gave an association constant, $K_a = 1.3 \times 10^8 \text{ M}^{-1}$ and $1.0 \times 10^8 \text{ M}^{-1}$. However, brain and skin cytosols from newborn rat were found to
differ in the relative amounts of Con A-non reactive AFP variant and contained 37% and 42% of this variant respectively. In view of these observations it appears that AFP in various feto-neonatal tissues of rat does not entirely represent fetal blood contamination and probably arises as a result of its independent synthesis in each tissue.

Studies on the distribution of AFP in feto-neonatal rat brain have revealed that it is distributed uniformly throughout the brain. Ontogeny of AFP in olfactory region, cerebrum, cerebellum and rest portions of the rat brain was similar to that in whole brain and it disappeared in these discrete regions after 7 d of postnatal development.

Presence of AFP was also demonstrated in human fetal brain. It was immunologically and electrophoretically identical to human fetal serum AFP. Developmental profile of AFP in human brain was distinct from that observed in serum compartment. It registered a peak level in brain at 20th week and declined rapidly to low levels after 24th week of gestation. It was absent (as measured by rocket assay) in the human fetal brain of subsequent ages. Unlike rodent AFP and like human AFP, it did not interact with estradiol. Brain AFP does not arise from fetal blood contamination since the blood contamination could account for traces of the AFP present in brain. Further ontogeny or AFP in brain is distinct from that observed in serum.
DEVELOPMENTAL CHANGES IN AFP AND ALBUMIN CONTENT IN SERUM OF RAT

Developmental profile of serum AFP exhibited an inverse relationship with age during postnatal life of rat. Serum AFP level declined after birth to 2.4% of the level observed in newborn rat, at 21st day of postnatal life. AFP could not be detected in sera of 28 d old rats by the present procedure. In contrast serum albumin elicited an increase by 37% during the same period. The albumin level rose in serum continuously to adult value of 37.5 mg ml$^{-1}$ in 9 weeks old rat.

SYNTHESIS AND SECRETION OF AFP AND ALBUMIN BY ISOLATED HEPATOCYTES IN CULTURE: CORRELATION OF SERUM AFP AND ALBUMIN PROFILES WITH THE LIVER CONTENT AND THEIR RATE OF SYNTHESIS IN THE HEPATOCYTES DURING DEVELOPMENT

AFP and albumin are synthesized in the hepatocyte and secreted into the serum. Serum level of these proteins undergoes striking change during fetoneonatal development.

Hepatocytes prepared from rat liver at various stages of development (18, 20 E and 1, 7, 14, 21, 28, 35 P and adult) by collagenase digestion procedure were maintained in short term (24 hrs) culture. Rate of synthesis and secretion of AFP and albumin were determined by following the incorporation of $[^{14}C]$-leucine into immunoprecipitable AFP and albumin by the hepatocytes cultured in leucine-free medium. Hepatocytes from newborn rat synthesized AFP at a
very high rate accounting for about 85% of the total protein synthesis while albumin synthesis was only 9%. Rate of AFP synthesis and secretion declined with the maturation of the hepatocytes. Hepatocytes from 21 d old rat demonstrated only 3% of the rate observed in fetal hepatocyte from 18 E rat. No synthesis of AFP could be demonstrated in the hepatocytes prepared from 4 week old rats. Serum AFP level and its content in liver also reduced to 1.3% and 4% respectively in 3 week old rat when compared to the values observed in 18 E fetal rats. It appears that age related change in serum AFP level and its content in liver is governed by the rate of AFP synthesis and secretion by the developing hepatocytes.

Maturation of the hepatocyte was accompanied by a marked increase in the rate of albumin synthesis, secretion and its intracellular content. Ratios of the rates of albumin to AFP synthesis in the hepatocytes from 18 E fetus and 1, 7, 14, 21, 28 d old neonatal rats were 0.05, 0.10, 0.28, 3.5 and 48 respectively. It would thus appear that an increase in serum albumin level and its intrahepatic content is due to a proportionate increase in the rate of albumin synthesis and secretion in the developing rat liver.

It may be concluded that differentiation and maturation of the hepatocyte is accompanied by a sequential inactivation of AFP and activation of albumin gene expression.
DEMONSTRATION OF AFP AND ALBUMIN SYNTHESIS IN DEVELOPING RAT BRAIN AND SKIN: CORRELATION OF TISSUE CONTENT OF AFP AND ALBUMIN WITH THEIR RATE OF SYNTHESIS

Dissociated mammalian brain cells and skin explants in primary cultures offers a useful system to investigate the biochemical events accompanying differentiation and growth of these tissues. Dissociated brain cells and skin explant cultures from newborn rat were used to answer the following questions: Is estradiol binding protein in developing rat brain and skin AFP? If so, where does this protein originate from?

Our observations, derived from $^{14}$C-leucine incorporation into immunoprecipitable AFP and albumin by dissociated brain cells and skin explant maintained in short term cultures, have provided evidence for the synthesis of AFP and albumin in brain and skin of newborn rat. Cycloheximide inhibited the linear incorporation of $^{14}$C-leucine into immunoprecipitable AFP and albumin as a function of time by these cultures. It strongly suggests that $^{14}$C-labelled AFP and albumin observed in brain cells and skin lysates (synthesis) and in medium (secretion) are constantly synthesized and secreted by these cultures. In addition, the intracellular content of both AFP and albumin in brain cells did not change significantly during the whole culture period. Moreover, these cells elicited a linear
accumulation (secretion) of the quantitable amounts of AFP and albumin into the medium. Total amounts of AFP and albumin secreted by brain cells were far in excess of their intracellular content at the initiation of the cultures. In view of these observations it was concluded that developing brain and skin represent an independent pool of AFP and albumin.

AFP and albumin secreted by newborn rat brain cells and skin explants cultures was immunologically, electrophoretically and with regards to molecular weights and estradiol binding properties similar to serum AFP and albumin. It may be concluded that genes for AFP and albumin in developing brain and for AFP in skin are also expressed in these tissues.

Rates of synthesis of AFP and albumin during feto-neonatal development of rat brain showed a continuous decline and their synthesis seems to be turned off after 7 d of postnatal life. High levels of these protein in fetal brain were due to their high rates of synthesis. Age related decrease in brain AFP and albumin reflected a proportionate decrease in the rates at which they are synthesized in this tissue.

Rate of synthesis of AFP in skin also decreased with increasing age and its synthesis is turned off after 14 d of postnatal life. A decrease in the skin AFP level can be accounted for by corresponding decline in the rate of its
HORMONAL CONTROL OF AFP AND ALBUMIN SYNTHESIS IN NEWBORN RAT LIVER, BRAIN AND SKIN

Effect of various hormones on the rate of AFP and albumin synthesis in the hepatocytes, brain cells and skin explant from newborn rats, was studied to gain some insight into ontogeny related regulation of synthesis of these proteins in rat tissues.

Dexamethasone was the most effective in inhibiting the rate of AFP synthesis and secretion in vitro cultures of hepatocytes (-45%), brain cells (-40%) and skin explant (-25%). It also caused an accelerated decrease in serum AFP level in vivo in newborn rats. dbcAMP also decreased the rate of synthesis and secretion of AFP in brain cells (-15%), hepatocytes (-7%) and skin explants (-4%). It also suppressed the serum AFP level in vivo in newborn rats supporting its in vitro effect.

Insulin exhibited a dual effect on the rate of AFP synthesis in various tissues. It increased AFP synthesis in hepatocytes (+21%) and demonstrated an opposite effect in brain cell (-15%) and skin explant (-4%). However, it did not affect serum AFP levels of newborn rats in vivo.

Neither did estradiol significantly affect the rate of AFP synthesis in various tissues in vitro nor did it change serum AFP level in vivo. The effects of dexamethasone, dbcAMP, insulin and estradiol on the rate of albumin
synthesis in vitro or its level in vivo were not significant. It appears that effects of these hormones are specific for AFP synthesis.

It appears that expression of AFP gene is a function of undifferentiated or less differentiated stem cells or embryonal cells. Different embryonal cell types with diverse ontogenic history do indeed synthesize AFP although the extent of synthesis is different. Thus expression of AFP and albumin genes during development offer a useful model for investigating molecular mechanism underlying tissue differentiation.