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Perinatal Changes of α-Fetoprotein Concentration in the Serum and its Synthesis in the Liver of Analbuminemic Rats[1]

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ABSTRACT

The profile of appearance and disappearance of α-fetoprotein (AFP) in the serum of analbuminemic rats, which have a genetically controlled lack of serum albumin, was studied. During the perinatal stage, AFP was present in the serum of analbuminemic rats, its concentration at birth being 10 mg/ml as in normal rats. In analbuminemic rats, the concentration of serum AFP remained at about 10 mg/ml during the first week after birth and then decreased rapidly during the next 2 weeks, becoming undetectable about 4 weeks after birth. In normal rats, the serum AFP concentration reached a maximum of 11.5 mg/ml at birth and then decreased sharply to an undetectable level within 4 weeks after birth, although a small rebound of AFP concentration was observed about 1 week after birth. AFP synthesis in analbuminemic and normal rats was examined by injecting [^{3}H]leucine i.p. and then measuring the radioactivity incorporated into the acid-insoluble fraction and immunoprecipitable fraction using anti-AFP antiserum. In analbuminemic rats, synthesis of AFP amounted to 7.5% of the total protein synthesis at birth and was maintained at about 7% of the total for the first week after birth and then decreased to 2% at 2 weeks after birth. In normal rats, AFP synthesis also amounted to 7.5% of the total protein synthesis at birth but decreased to about 2% at 2 days after birth and then remained at a low level for about 2 weeks. In both normal and analbuminemic rats, AFP synthesis was undetectable at 4 weeks after birth. These data show that AFP synthesis is shutoff after birth irrespective of the serum albumin concentration during neonatal development.

INTRODUCTION

A strain of rats with genetically controlled lack of serum albumin (analbuminemic rats) was established (9), and the trait was shown to be autosomally recessive (10). The mutant rats show normal longevity, fertility, and other general characters (9, 10) except for slight growth retardation and hyperlipidemia (1, 9). Despite the absence of serum albumin, the total serum protein concentration of this mutant is the same as that of normal rats (5), because the absence of serum albumin is compensated for by increases in the concentrations of globulin fractions, especially transferrin, α_1-x-glycoprotein, α_2-macroglobulin, and γ-globulin (5). Therefore, this analbuminemic mutant seems to be a good model for use in studies on the biological roles of serum albumin and the biological correlations between serum albumin and other serum proteins.

AFP[3] is a representative oncofetal serum protein (18), which has been shown to have a very similar primary structure to that of serum albumin (6, 14) and to share the antigenicity of the latter under denatured conditions (13). Moreover, the regulations of the productions of AFP and serum albumin are thought to be closely related (7, 15). To obtain more information on the correlation between the synthesis of these 2 related proteins, we examined the perinatal changes of AFP synthesis in analbuminemic rats.

MATERIALS AND METHODS

Animals. Analbuminemic rats were derived from JCL-Sprague-Dawley rats and are maintained in Sasaki Institute, Tokyo, Japan. Normal Sprague-Dawley rats were obtained from the stock of Sprague-Dawley rats of CLEA Japan, Kanagawa, Japan, in which the original analbuminemic rat was found.

Rat AFP and Anti-AFP Antiserum. Rat AFP was purified from the amniotic fluid of Sprague-Dawley rats on the 18th day of pregnancy. Amniotic fluid was dialyzed against 10 mM potassium phosphate buffer (pH 7.3) containing 150 mM NaCl and applied to a CM-Affi-Gel Blue column. AFP passed through the column, but most of the serum albumin was removed by this procedure. The fractions containing AFP were applied to a DEAE-cellulose column after dialysis against 10 mM potassium phosphate buffer (pH 7.3) containing 50 mM NaCl. AFP was eluted with 10 mM potassium phosphate buffer containing 200 mM NaCl and was further purified by passage through an ultrafiltration membrane XM 100A. The resulting preparation of AFP gave a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Anti-rat AFP antiserum was prepared by injecting purified AFP with Freund's complete adjuvant into rabbits. The anti-rat AFP antiserum obtained after several booster injections gave a single precipitin line on immunoelectrophoresis against newborn rat serum and amniotic fluid but no precipitin line against adult rat serum (data not shown).

Two-Dimensional Gel Electrophoresis of Newborn Rat Serum. The serum protein of newborn rats was analyzed by 2-dimensional gel electrophoresis as described previously (5). Protein was located by staining with 0.1% Coomassie Brilliant Blue R-250. AFP and serum albumin were identified by the method of Showe *et al.* (19) using anti-AFP and anti-rat serum albumin antisera, respectively.

Assays of Serum AFP and Serum Albumin. AFP and serum albumin were determined by single radial immunodiffusion (8) using anti-rat AFP and anti-rat serum albumin antisera, respectively. Anti-rat serum albumin was prepared as described previously (4).

Determination of the Amount of AFP Synthesis in the Liver. The amount of AFP synthesis relative to total protein synthesis was determined by labeling newly synthesizing protein with [^{3}H]leucine. [^{3}H]-Leucine (50 μCi/rat) was injected i.p., and the liver was excised 15 min later while acid-insoluble radioactivity was still not detectable in the serum. The liver was promptly homogenized in a mixture of 1% sodium deoxycholate and 1% Triton X-100, and the homogenate was centrifuged at 105,000 × *g* for 60 min. Total protein synthesis was determined by measuring acid-insoluble radioactivity in the resulting

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[3] The abbreviation used is: AFP, α-fetoprotein.

supernatant by the method described previously (4). AFP synthesis was determined by immunoprecipitation of AFP with anti-AFP antiserum after determination of the concentration of AFP in the extract. In the immunoprecipitation assay, the AFP concentration was adjusted to exactly 100 μg/ml. The immunoprecipitate was washed 3 times with a solution containing sodium deoxycholate and 1% Triton X-100 and then dissolved in Protosol for measurement of its radioactivity.

Chemicals. L-[^{3}H]Leucine (113.8 Ci/mmol) and Protosol were purchased from New England Nuclear, Boston, Mass. CM-Affi-Gel Blue was a product of Bio-Rad Laboratories, Richmond, Calif. DEAE-cellulose was a product of Whatman, Inc., Maidstone, Kent, England. Freund's complete adjuvant was purchased from Difco Laboratories, Inc., Detroit, Mich. Ampholine was a product of Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals were reagent grade.

RESULTS

Two-Dimensional Gel Electrophoresis of Serum Proteins at Birth. The total serum protein concentration of analbuminemic rats at birth was 24 mg/ml, which was very similar to that of normal rats (23 mg/ml) in spite of the absence of serum albumin (Fig. 1). Two-dimensional gel electrophoresis showed the complete absence of serum albumin with compensatory increases of serum proteins such as transferrin and α_2-macroglobulin in analbuminemic rat serum (Fig. 1). Since the serum albumin concentration of normal newborn rats was about 5 mg/ml, the increased amounts of serum proteins other than albumin in analbuminemic rats amounted to about 25% of the total serum protein.

Serum AFP Concentration. Two-dimensional gel electrophoresis showed that at birth the most abundant protein in the serum of both normal and analbuminemic rats was AFP. We compared the AFP concentrations in the 2 types of rats during the neonatal period. As shown in Chart 1, before birth, the AFP concentration in analbuminemic rats was similar to that in normal rats in spite of the absence of serum albumin. In both analbuminemic and normal rats, the AFP concentration increased to about 10 mg/ml at birth. In analbuminemic rats, the concentration was then maintained at about 10 mg/ml for 1 week whereas in normal rats the AFP concentration decreased rapidly after birth. Thus, the AFP concentration of analbuminemic rats was about twice that of normal rats during the first

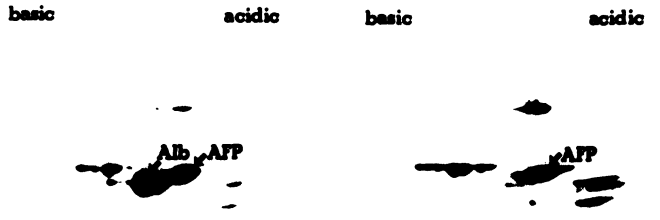

Fig. 1. Two-dimensional gel electrophoresis of serum proteins from normal and analbuminemic rats at birth. One μl of serum was subjected to 2-dimensional gel electrophoresis by the method of O'Farrell (11). After electrophoresis, proteins were located by staining with Coomassie Brilliant Blue R-250. AFP and albumin were identified by their molecular weights and isoelectric points on the gel. They were further confirmed by extracting the spots and subjecting them to double immunodiffusion against anti-AFP antiserum and antialbumin antiserum. *Left*, normal Sprague-Dawley rat serum; *right*, analbuminemic rat serum. *Alb*, albumin.

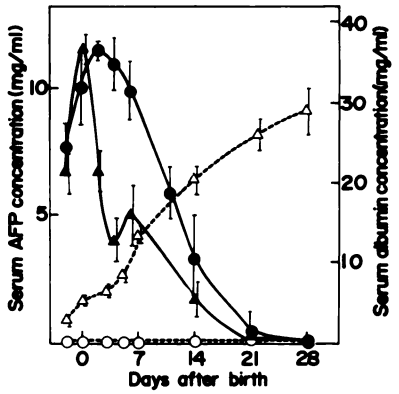

Chart 1. Serum concentrations of AFP and albumin in normal and analbuminemic rats during the perinatal period. Six rats at each stage of development were sacrificed, and their sera were collected. The concentrations of AFP and albumin were determined by single radial immunodiffusion. ▲, AFP concentration in normal Sprague-Dawley rats; ●, AFP concentration in analbuminemic rats; △, albumin concentration in normal Sprague-Dawley rats; ○, albumin concentration in analbuminemic rats. *Points*, means for 6 rats; *bars*, S.D.

3 weeks after birth. However, although there was a clear difference in the AFP concentrations in normal and analbuminemic rats after birth, the most significant finding was that, even in analbuminemic rats which lack serum albumin, the AFP concentration decreased to an undetectable level within 3 to 4 weeks after birth (Chart 1).

AFP Synthesis in the Liver. Next we examined the capacities of the liver of analbuminemic and normal rats to synthesize AFP after birth. The amount of AFP synthesis relative to total protein synthesis was measured by immunoprecipitation using anti-rat AFP antiserum as described in "Materials and Methods." The specificity of the immunoprecipitation assay was tested by dissolving the precipitate in 125 mM Tris-HCl buffer (pH 6.8) containing 0.1% sodium dodecyl sulfate and 17 mM 2-mercaptoethanol and subjecting it to electrophoresis in duplicate on 10% polyacrylamide gel. After electrophoresis, one lane was stained with Coomassie Brilliant Blue R-250 and the other was cut into 3-mm-thick slices for measurement of radioactivity. The results in Chart 2 clearly demonstrate that the immunoprecipitation method is specific for AFP.

AFP synthesis in the liver was measured as described above. As shown in Chart 3, in both analbuminemic and normal rat livers, AFP synthesis at birth amounted to about 7.5% of the total protein synthesis. In analbuminemic rat liver, AFP synthesis continued to be about 7% of the total protein synthesis for the first week after birth but decreased rapidly during the second week. In normal rats, AFP synthesis decreased to 2% of the total in the first 2 days after birth, increased slightly after about 1 week, and then decreased gradually. We estimated only the amount of AFP synthesis relative to total protein synthesis and did not measure the pool sizes of leucine in the liver of the 2 strains or their total amounts of protein synthesis. However, it is reasonable to compare the relative amounts of AFP synthesis in the 2 strains, because during the perinatal stage their total serum protein concentrations and increases in body weight were similar.

DISCUSSION

The present study clearly showed that shutoff of the AFP gene took place irrespective of the serum albumin concentra-

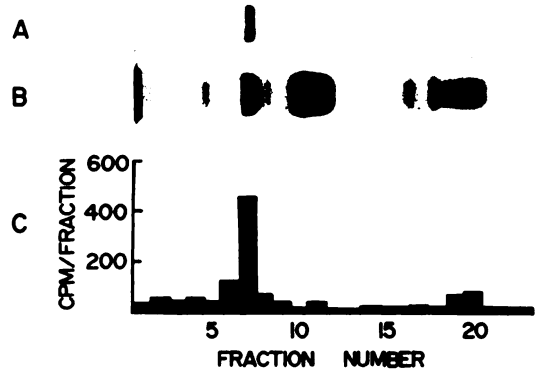

Chart 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of immunoprecipitate. Liver extract of normal Sprague-Dawley rats labeled with [^{3}H]leucine at birth was immunoprecipitated with anti-AFP antiserum. The precipitate was dissolved in a small volume of sample buffer and subjected to electrophoresis on 10% polyacrylamide gel in duplicate in parallel with purified rat AFP. A lane of purified AFP (*A*) and one of the duplicate lanes of immunoprecipitate (*B*) were stained with Coomassie Brilliant Blue R-250. The other duplicated lane was cut into 3-mm-wide slices and assayed for radioactivity (*C*).

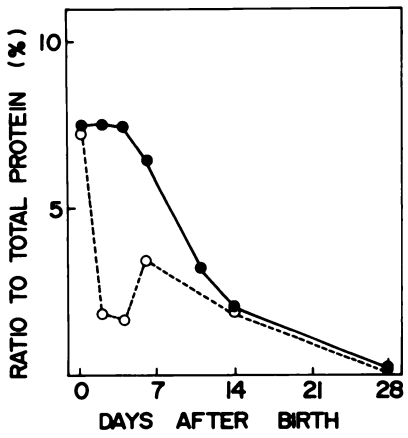

Chart 3. *In vivo* AFP synthesis in the livers of normal Sprague-Dawley rats and analbuminemic rats. *In vivo* AFP synthesis was assayed as described in "Materials and Methods." *Points*, means for 3 rats. ●, analbuminemic rats; ○, normal Sprague-Dawley rats.

tion during development of newborn rats. Because of the clear reciprocal relationship between the serum AFP and albumin concentrations during normal neonatal development, it has been proposed in earlier works that there must be some interaction between the expressions of the genes for AFP and albumin (7, 15). Moreover, AFP was synthesized by liver cells during chemical damage or carcinogenesis of the liver and during regeneration of the liver when albumin synthesis decreased (16, 17). Therefore, we expected that, in analbuminemic rats, AFP would persist for a long time after birth. But this was not the case. In human cases of analbuminemia also, serum AFP was reported to be under the detectable level even in the neonatal stage (2, 3).

The present results on AFP synthesis suggest that shutoff of the AFP gene is slightly retarded in analbuminemic rats with resulting retardation of the decrease in serum AFP concentration. We do not know the reason for this slight delay in decrease of AFP synthesis in analbuminemic rats. Possibly there is some machinery that recognizes the total protein concentration of serum and activates some gene for serum proteins. In mice, the rate of decrease in serum AFP concentration during normal neonatal development has been reported to be genetically controlled (12). Genetic studies on this problem are now in progress.

We did not exclude the possibility of some interrelation of serum protein concentration and AFP gene expression, but this work clearly showed that shutoff of the AFP gene principally occurred irrespective of the serum albumin concentration during neonatal development.

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