Subcellular Distribution and Molecular Heterogeneity of α_1-Fetoprotein in Newborn Rat Liver†

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ABSTRACT: Livers from 12 day old rats were subfractionated into nuclei, mitochondria, polyribosomes, rough microsomes, smooth microsomes, Golgi complex, and plasma membranes. The purity of the subcellular fractions was established from the specific activities of marker enzymes. The concentrations of α_1-fetoprotein (AFP) were found to be highest in rough and smooth microsomes and in the Golgi complex. The ratio of albumin to AFP in serum was 15.8 to 1, whereas the ratio was a constant 7.3 to 1 for each of the secretory organelles; this 2.2-fold difference between the albumin/AFP ratios of serum and subcellular fractions seems referable to a 2.2-fold difference in their serum half-lives. These observations indicate that AFP follows the secretory pathway generally recognized for liver export proteins [Palade, G. (1975) *Science 189*, 347–358] and point to parallel dynamics of secretion of AFP and albumin. Polysomal AFP did not interact with concanavalin A (Con A)–Sepharose, whereas rough microsomal AFP was 93% Con A reactive. The Con A reactivity of AFP in the Golgi complex and plasma membranes was similar to that of serum AFP (62% reactive). Rough microsomal AFP had a slower electrophoretic mobility than serum AFP. These observations can be explained by sequential glycosylation reactions occurring in subcellular compartments during secretion. Our data permit the drawing of a scheme for the processing of AFP in the liver cell and provide new insight into the biochemical significance of AFP molecular variants. They also indicate that, in the rat, the subcellular liver functions for the production, secretion, and processing of export peptides develop into an adult-type pattern early in the postnatal development.

Albumin and α_1-fetoprotein (AFP[1]) are two major liver parenchymal cell products, with physicochemical, functional, and structural similarities (Ruoslahti & Engvall, 1976). Albumin in the adult and AFP in the embryo are the dominant export products of the liver; in the perinatal period, their production is complementary (Gitlin & Gitlin, 1975; Tamaoki et al., 1974). AFP and albumin thus form a powerful combination of markers for studies on liver physiology and differentiation.

One aim of current research on AFP is to identify the subcellular structures involved in its synthesis, processing, and secretion, as compared with albumin. Most AFP studies conducted so far have been based on electron microscope immunolocalization analyses. Although useful, this method provides only qualitative and limited information and at times has yielded conflicting results. As an alternative approach, we have performed direct determinations of the AFP content of isolated newborn rat liver cell organelles, in comparison with that of albumin. In addition, we have analyzed subcellular fractions in regard to the molecular heterogeneity of AFP; as a glycopeptide with reactivity toward carbohydrate-binding lectins (Smith & Kelleher, 1973; Kerckaert et al., 1977), AFP was particularly well suited to evaluate the sequential processing of an export peptide in subcellular compartments.

Our results indicate a similar subcellular distribution and parallel dynamics of secretion of AFP and albumin. They also provide further insight into the biochemical significance of AFP molecular variants and new information on peptide export functions of the neonatal liver.

Experimental Procedure

Animals. Twelve day old Sprague-Dawley rats were used. At this age, the liver is ultrastructurally well developed and active in the production of both albumin and AFP (Dallner et al., 1966; Bélanger et al., 1975; Guillouzo et al., 1976).

Isolation of Purified Subcellular Fractions. The rats were killed by decapitation, between 9 and 10 a.m.; their livers were removed within 1 min, kept in ice cold 0.25 M sucrose, and homogenized within 20 min. Livers from 60 to 80 rats were pooled, homogenized, and fractionated into nuclei, mitochondria, rough microsomes, smooth microsomes, Golgi apparatus, and plasma membranes, as described previously for adult rat livers (Fleischer & Kervina, 1974). Protein concentrations were determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin (BSA) (Armour Pharmaceutical Co.) as a standard. Phosphorus was determined by a modification of the method of Chen et al. (1956), as described in Rouser & Fleischer (1967). Marker enzymes were used to estimate the degree of contamination of the fractions with other subcellular organelles. Rotenone-insensitive NADH-cytochrome *c* reductase and glucose 6-phosphatase activities were used to estimate the degree of contamination with endoplasmic reticulum (Fleischer & Fleischer, 1967; Van Golde et al., 1971). Contamination with plasma membranes, mitochondria, and Golgi complex were estimated respectively with 5′-nucleotidase (Widnell & Unkeless, 1968), succinate-cytochrome *c* reductase (Fleischer & Fleischer, 1967), and UDP-Gal:*N*-acetylglucosamine galactosyltransferase (Fleischer, 1974) activities.

For AFP and albumin analyses, the subcellular fractions were solubilized in 2% sodium deoxycholate with ten strokes of a tight glass pestle in a Dounce homogenizer and dialyzed

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[1] Abbreviations used: AFP, α_1-fetoprotein; PBS, phosphate-buffered saline, 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.2; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; Con A, concanavalin A.

0006-2960/79/0418-1962$01.00/0

for 48 h at 4 °C against six changes of PBS (phosphate-buffered saline, 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.2). The preparations were used for AFP and albumin measurements and for AFP variants characterization by chromatography on Con A–Sepharose. AFP and albumin were also isolated from rough microsomes by immunoadsorption column chromatography and characterized by polyacrylamide gel electrophoresis.

Isolation of Polysomes. Total polysomes were isolated by the sucrose gradient method (Palacios et al., 1972) from pools of 20 livers. They were dialyzed for 6 h against PBS, then adjusted to 0.1 M EDTA, and dialyzed again for 48 h at 4 °C against six changes of PBS. They were used for AFP characterization by Con A–Sepharose chromatography.

Antigens and Antibodies. Preparations of pure rat AFP and pure rat albumin were initially obtained from a pool of amniotic fluid by a combination of gel filtration, lectin-affinity chromatography, isoelectric focusing, or preparative polyacrylamide gel electrophoresis and served to raise antisera in sheep (Bélanger & Dufour, 1974; Bélanger, 1975). The antigens were subsequently purified either by the immunoprecipitation method of Nishi (1970) (AFP) or by immunoadsorption column chromatography (AFP and albumin). For the latter procedure, the IgG fraction of the sheep antisera was purified to homogeneity by 33% ammonium sulfate precipitation and anion-exchange chromatography on QAE-Sephadex A-50 (Bélanger et al., 1976) and coupled to preactivated CNBr-Sepharose (Pharmacia), at the ratio of 25 mg of protein per g of Sepharose and following the procedure recommended by the manufacturer. Source materials (normal adult rat serum for albumin, fetal rat serum for AFP) were dialyzed against PBS and chromatographed on IgG-Sepharose columns equilibrated in the same buffer. The adsorbed material was eluted with 0.1 M glycine–HCl, pH 2.0, neutralized with 0.5 M Na_2HPO_4, dialyzed against PBS, and rechromatographed. The purity of the antigen preparations was assessed by polyacrylamide gel electrophoresis and by double immunodiffusion and immunoelectrophoresis against anti-normal adult rat and sheep serum antisera. In case of residual contamination, final purification was achieved through one or a combination of the physicochemical methods mentioned above. Pure AFP and albumin preparations served to raise high titer anti-AFP sera in rabbits and anti-albumin sera in goats. The monospecificity of these antisera was confirmed by double immunodiffusion against normal fetal and adult rat sera.

AFP and Albumin Measurements. Albumin was measured by electroimmodiffusion with the use of goat antiserum, against standards of normal adult rat serum with albumin content determined on a Du Pont automatic clinical analyzer (bromocresol green dye binding method). Dilutions of test and standard samples were made in PBS. The sensitivity of the assay was 1 μg/mL. For AFP, a double antibody radioimmunoassay was developed. AFP was radioiodinated as follows (Ruoslahti & Seppala 1971). Five micrograms of pure antigen in 2 μL of PBS was mixed with 10 μL (1 mCi) of carrier-free $Na^{125}I$ (Amersham/Searle Corp.). One hundred micrograms of Chloramine-T (Eastman Organic Chemicals) in 25 μL of 0.5 M sodium phosphate, pH 7.0, was added and the reaction allowed to proceed for 10 s. Sodium bisulfite (250 μg) in 100 μL of sodium phosphate buffer was then added, followed by 4 mg of potassium iodide in 200 μL of distilled water. The reaction mixture was chromatographed on a Sephadex G-25 column equilibrated in PBS, and the labeled AFP, separated from the unreacted isotope, was stabilized in 2% BSA in PBS.

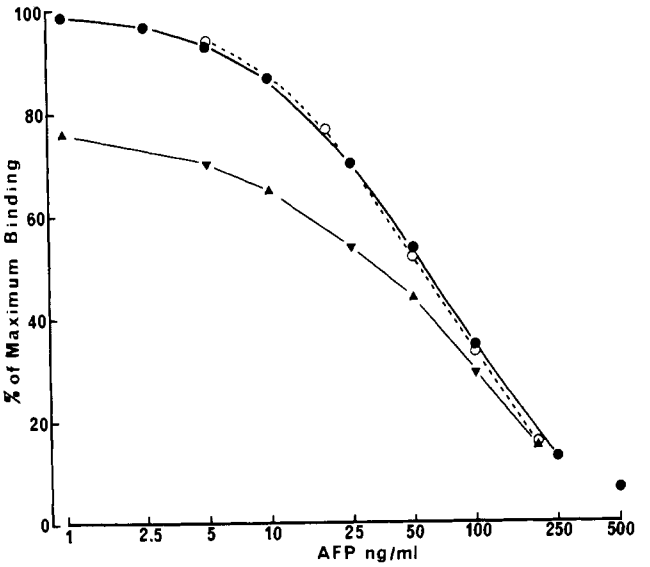

FIGURE 1: Radioimmunoassay of rat AFP. Means of triplicate dose–response points with two sets of standards, ours (●) and Dr. Stewart Sell's (○), and with purified AFP added to a pool of normal adult rat serum (▲) (amounts of AFP added given by the abscissa scale). By reference to the standard curve, the analytical recovery of AFP in adult rat serum yields at all points a basal AFP serum level of 20–25 ng/mL, indicative of the accuracy of the system. For this particular experiment, a 24-h equilibrium assay was used; higher sensitivity can be achieved with sequential saturation procedures (Bélanger et al., 1976).

Equilibrium and sequential saturation assays were set up under conditions similar to those developed for the double antibody radioimmunoassays and enzyme immunoassays for human AFP (Bélanger et al., 1976). Rat AFP standards were prepared from stocks of purified antigen, quantitated by the procedure of Lowry et al. (1951). Dilutions of test and standard samples were in 1% BSA in PBS. The sensitivity limit of the assay was 3 ng of AFP/mL (Figure 1).

Affinity Chromatography on Con A–Sepharose. Detergent-solubilized subcellular fractions, EDTA-treated polysomes, and serum samples were dialyzed for 48 h at 4 °C against six changes of 50 mM Tris-HCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, 1 M NaCl, pH 7.6 (starting buffer). The chromatography was performed at room temperature. Three-milliliter columns of packed Con A-Sepharose (Pharmacia) were washed with 30 mL of starting buffer. The samples, adjusted in starting buffer to no more than 5 μg of AFP (the AFP binding capacity of the columns was at least 50-fold higher, as tested with fetal serum), were applied to the columns in 1-mL aliquots (except for polysomal preparations, 3 mL) and allowed to stand in contact with the matrix for 15 min before elution. The columns were then eluted in 3-mL fractions with 15–18 mL of starting buffer (Con A nonreactive fraction) followed by 18–21 mL of 50 mM Tris-HCl, pH 7.6, containing 0.4 M α-methyl D-mannopyranoside (Aldrich Chemical Co.) (Con A reactive fraction). AFP was assayed on the starting samples, the individual elution fractions, and the pooled total Con A reactive and Con A nonreactive fractions (controls showed that neither of the elution buffers affects the analytical recovery of AFP in the radioimmunoassay).

Polyacrylamide Gel Electrophoresis. Ten percent polyacrylamide gel electrophoresis was performed according to the standard gel system of Ornstein (1964) and Davis (1964). Microsomal and serum AFP and albumin, isolated by immunoadsorption, were analyzed in 3–10-μg samples. Coomassie Blue stained gels were scanned at 650 nm on a Gilford spectrophotometer.

Table I: Characterization of Subcellular Fractions of Neonatal Rat Livers by Using Marker Enzymes[a]

fraction	bound P (μg/mg of protein)	Glu-6-Pase	RI NADH cyt *c* red.	succinate cyt *c* red.	5'AMP'ase	Gal transf.
homogenate	21.0	0.040	0.231	0.077	0.049	10.5
mito	11.8	0.000	0.154	0.234	0.023	0.3
Golgi complex	30.6	0.046	0.190	0.008	0.127	523
rough micros	46.9	0.172	0.746	0.004	0.035	10.2
smooth micros	35.6	0.175	1.07	0.007	0.262	57.6
PM	24.2	0.057	0.220	0.036	1.21	4.5
nuclei	46.6	0.024	0.074	0.004	0.023	3.3

[a] Specific activities are in μmol min^{-1} (mg of protein)$^{-1}$ except for galactosyltransferase in μmol h^{-1} (mg of protein)$^{-1}$. Reactions were at 30 °C except for galactosyltransferase at 37 °C. Values are the average of two preparations except for nuclei, plasma membranes, and mitochondria which are for one preparation. Abbreviations used: P, phosphorus; Glu-6-Pase, glucose 6-phosphatase; RI NADH cyt *c* red., rotenone-insensitive NADH-cytochrome *c* reductase; succinate cyt *c* red., succinate-cytochrome *c* reductase; 5'AMP'ase, 5'-nucleotidase; Gal transf., galactosyltransferase; mito, mitochondria; micros, microsomes; PM, plasma membrane.

Carbohydrate Determinations. The carbohydrate composition of rat AFP and its Con A reactive and Con A nonreactive variants was determined by gas–liquid chromatography according to Chambers & Clamp (1971). The preparations of AFP were from fetal rat serum, the method of purification was that of Nishi (1970), and the chromatography on Con A–Sepharose was as described above. Upon rechromatography on Con A–Sepharose, the purified AFP variants maintained over 90% of their reactive or nonreactive character, indicating minimal cross-contamination.

Results

Characterization of Purified Subcellular Fractions. The procedure developed for the isolation of subcellular fractions from adult rat livers was found to be directly applicable to livers from twelve day old rats. The fractions showed a degree of enrichment of marker enzyme activities similar to that found in adult rat livers (Fleischer & Kervina, 1974) (Table I) and, thus, were of comparable purity. The contamination of the Golgi apparatus fraction by endoplasmic reticulum was about 18%, as estimated from the glucose 6-phosphatase and rotenone-insensitive NADH-cytochrome *c* reductase activities of the Golgi fraction compared with those of the rough microsomes.[2] Rough microsomes showed little contamination with Golgi complex, approximately 2% as measured by the galactosyltransferase activity of that fraction. The smooth microsomes, however, were a rather heterogeneous fraction, containing about 10% Golgi complex, using galactosyltransferase activity as marker enzyme for the Golgi complex, and about 20% plasma membranes, as measured by the 5'-nucleotidase activity as marker enzyme for plasma membranes. The latter value is a slight overestimate since smooth endoplasmic reticulum contains some 5'-nucleotidase activity as shown by cytochemical localization (Widnell, 1972).

Isolation of Polysomes. Polysomes were extracted from newborn livers with a yield of 15 A_{260} units per g of tissue and had a typical A_{260}/A_{280} ratio of 1.80. Their structure was well preserved, as shown by their sedimentation profiles on linear sucrose gradients and their specific binding of ^{125}I-labeled anti-AFP antibodies (Bélanger et al., 1979). Possible contamination of polysomal AFP preparations by nonpolysomal AFP was evaluated by carrying out the polysome extraction procedure in the presence of [^{125}I]AFP (10^4 cpm per mL of tissue homogenization buffer). The radioactivity, 0.01%, recovered in the final polysomal AFP preparation, yielded an estimated 15% contamination of polysomal AFP by nonpolysomal AFP, from the AFP content of tissue homogenate vs. polysomal preparation.

Table II: AFP and Albumin Content of Liver Subcellular Fractions and Sera of Neonatal Rats[a]

fraction	AFP[a]	albumin[a]	ratio
mito	0.034	0.24	7.3
nuclei	0.060	<0.1[b]	
rough micros	0.781	5.7	7.3
smooth micros	1.80	12.9	7.2
Golgi complex	8.01	60.2	7.5
PM	0.195	1.4	7.0
serum	728	11500	15.8

[a] Results are in μg/mg of protein, except for sera in μg/mL. Average of two preparations. [b] Below detection limit. For abbreviations, see footnote *a* to Table I.

AFP and Albumin Concentration in Purified Subcellular Fraction. AFP and albumin were measured on two series of organelle preparations, with comparable results. The data, presented in Table II, show a selective concentration of AFP and albumin in secretory organelles, with maximum specific activities in the Golgi complex. The albumin/AFP concentration ratio in liver organelles was remarkably constant and less than half that of serum. The albumin values for rough microsomes, smooth microsomes, and Golgi complex were virtually identical with those reported by Peters et al. (1971) for normal adult rat liver (6.4, 12.1, and 59.5 μg/mg of protein, respectively).

Analysis of AFP Molecular Variants on Con A–Sepharose. The Con A–Sepharose chromatography profiles of serum and subcellular AFP preparations are presented in Figure 2. The patterns point to a virtual absence of Con A reactive AFP in the polysome preparations. In contrast, the organelle preparations all show a dominance of Con A reactive over Con A nonreactive AFP populations, most prominent in rough microsomes (ratio Con A nonreactive/Con A reactive of 0.24), and relatively uniform in smooth microsomes (ratio 0.59), Golgi complex (0.51), and plasma membranes (0.58); serum AFP in turn appears predominantly Con A nonreactive (ratio 1.3).

To verify whether any of the tested preparations could modify the intrinsic Con A–Sepharose binding behavior of AFP, control chromatographies were performed with [^{125}I]AFP added as an indicator to all serum, polysome, and organelle samples assayed (2×10^5 cpm per 1-mL sample).

[2] The enzyme specific activity of rough microsomes must be multiplied by 3/2 to obtain the activity of endoplasmic reticulum membranes stripped free of ribosomes (Fleischer & Kervina, 1974): the percent contamination of the Golgi fraction by endoplasmic reticulum membranes on a protein basis therefore is $[0.046/(0.172 \times 3/2)] \times 100 = 18$. A similar figure is obtained if the calculation is made on the basis of rotenone-insensitive NADH-cytochrome *c* reductase activities (i.e., $[0.190/(0.746 \times 3/2)] \times 100 = 17$).

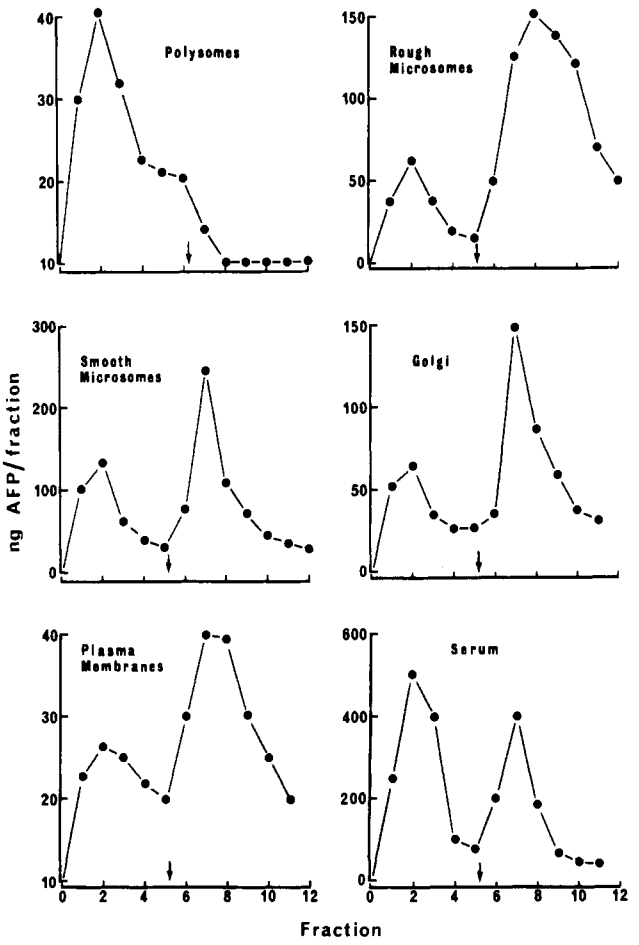

FIGURE 2: Con A–Sepharose chromatography of AFP from serum and liver subcellular fractions of neonatal rats. Arrows indicate change from starting buffer to α-methyl D-mannopyranoside containing buffer. Average points of duplicate chromatographies on two series of samples.

Table III: Con A–Sepharose Reactive AFP (Fraction of Total AFP) in Serum and Liver Subcellular Fractions of Neonatal Rats

fraction	Con A reactive AFP[a] (%)
polysomes	12
rough micros	85
smooth micros	70
Golgi complex	65
PM	63
serum	61

[a] Average of triplicate determinations on two series of samples. For abbreviations, see footnote *a* to Table I.

The results showed indeed that the amount of Con A reactive [^{125}I]AFP recovered from the columns varied widely depending on the carrier sample: a fraction of Con A reactive [^{125}I]AFP remained uneluted (i.e., "irreversibly" adsorbed to the column) which varied from 6% to as much as 32% of total amount of [^{125}I]AFP applied. The fraction of Con A nonreactive [^{125}I]AFP recovered from the columns was, however, quite constant, varying only from 31% to 36% of the total amount of [^{125}I]AFP applied. Therefore, a more accurate estimation of the actual proportion of total Con A reactive AFP in the various samples tested could be obtained by subtracting the amount of Con A nonreactive AFP recovered from the total amount of AFP applied, as was previously recommended by Smith et al. (1977). These data are presented in Table III. They corroborate that the proportion of Con A reactive AFP progresses from lowest in polysomal preparations (12%) to highest in rough microsomes (85%), but they also indicate that this proportion is actually maintained at approximately 62% in Golgi complex (the measured 65% is a slight overestimate because of contamination by endoplasmic reticulum) and plasma membranes, as well as in serum. The proportion of Con A reactive AFP in smooth microsomes is intermediate between that in rough microsomes and that in Golgi complex/plasma membranes/serum; the measured 70% is an underestimate because of contamination by Golgi complex and plasma membranes.

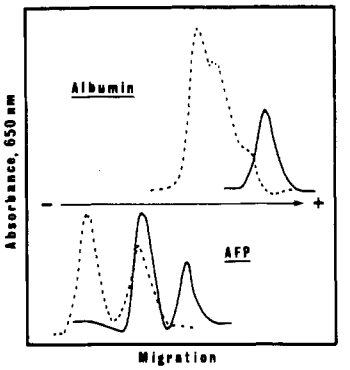

FIGURE 3: Relative electrophoretic mobilities (10% polyacrylamide gel electrophoresis under nondenaturing conditions) of albumin and AFP isolated by immunoadsorption column chromatography from serum (solid lines) and liver rough microsomes (dashed lines) of neonatal rats.

Table IV: Carbohydrate Composition of Rat AFP and Its Con A Reactive and Con A Nonreactive Variants

residue	% carbo[a] total AFP	molar ratios[b] Con A reactive	molar ratios[b] Con A nonreactive
mannose	0.9	1.0	1.0
N-acetyl-glucosamine	1.0	0.9	1.1
galactose	0.4	0.3	1.1
sialic acid	0.7	0.1	0.5

[a] Percent carbohydrate based on mol wt 70000; average of duplicate determinations on three samples. [b] Based on 1 mannose; average of duplicate determinations on two preparations.

Polyacrylamide Gel Electrophoresis of Microsomal and Serum AFP and Albumin. Both AFP and albumin isolated from rough microsomes migrated slower than their serum counterpart (Figure 3). Whereas serum albumin migrated as a single symmetrical peak, microsomal albumin appeared to be a composite of three subfractions. Both serum and microsomal AFP were distinctly separated into two fractions, with little difference in the ratios between the slow and fast variants (respectively 63/37 and 59/41).

Carbohydrate Composition of AFP. The carbohydrate composition of fetal rat serum AFP, and its Con A reactive and Con A nonreactive variants, is presented in Table IV. Rat AFP contains 3% carbohydrate, and the Con A nonreactive variant has a molar ratio of galactose + sialic acid to mannose + *N*-acetylglucosamine more than threefold higher than that of the Con A reactive variant.

Discussion

The data produced in this work indicate that the subcellular distribution of AFP in normal neonatal liver is similar to that of albumin. They confirm previous evidence from biochemical and electron microscopic immunolocalization observations, pointing to AFP and albumin locations restricted to membrane-bound polysomes, rough and smooth endoplasmic reticulum, and Golgi apparatus (Glaumann & Ericsson, 1970;

Peters et al., 1971; Redman & Cherian, 1972; Kanai et al., 1974; Koga & Tamaoki, 1974; Guillouzo et al., 1975, 1976, 1978; Shikata, 1975; Peyrol et al., 1977; Peters, 1977; Bergeron et al., 1978). They also extend the results of a similar study on albumin distribution in adult rat liver (Peters et al., 1971), to include the plasma membrane fraction. Thus, AFP appears to follow the secretory pathway generally recognized for albumin and other liver export peptides (Palade, 1975). One implication, with regard to its estrogen-binding properties in certain species (Nunez et al., 1971), is that AFP is unlikely to act in the hepatocyte as a soluble cytoplasmic hormone receptor-like molecule, as it possibly does in other cell types (Michel et al., 1974; Attardi & Ruoslahti, 1976); it might, however, exert such functions in secretory organelles (Aussel & Masseyeff, 1976) or in plasma membranes.

Our measurements establish a constant relationship between the amounts of AFP and albumin present in the secretory organelles. This observation, and the similar transit times of AFP and albumin ($\simeq$15 min) (Peters et al., 1971; Peters & Peters, 1972; Bélanger et al., 1975), indicates that the two proteins have a parallel dynamic flow through the secretory channels, even if AFP may have to be attached to the endoplasmic reticulum membrane for glycosylation, while albumin is not glycosylated and not membrane bound (Redman & Cherian, 1972). Our data also show a 2.2-fold difference in the albumin/AFP ratios of serum vs. subcellular organelles (15.8 vs. 7.3), which coincides with a half-life of serum albumin 2.2 times longer than that of AFP [2.2 days (Munro & Downie, 1964; Sell, 1974; Yeoh & Morgan, 1974) vs. 1 day (Sell, 1974; Colquhoun et al., 1974; Bélanger et al., 1975)]. With similar dynamics of secretion, and intracellular levels proportional to serum levels normalized for half-life values, the following conclusions can also be reached: the overall body fluid compartmentation of AFP and albumin is similar; their intracellular level gives a direct estimate of their relative rates of synthesis; the relationship between AFP and albumin serum levels is determined only by their respective rates of synthesis and catabolism.

The carbohydrate composition of rat serum AFP (Table IV; Kerckaert et al., 1977; Watabe, 1974) reveals only two residues with Con A binding specificity: mannose and *N*-acetylglucosamine (Sharon & Lis, 1972). These residues are therefore presumably responsible for the binding of AFP to Con A; further, the higher proportion of galactose and sialic acid in the Con A nonreactive AFP may limit the availability of mannose and *N*-acetylglucosamine and account for the lack of reactivity to Con A. In the light of current knowledge on the structure of glycoproteins and their sequential glycosylation in the liver (Schachter, 1974; Waechter & Lennarz, 1976; Beeley, 1974; Kornfeld & Kornfeld, 1976; Clamp, 1975), two results of the affinity chromatography of AFP in subcellular fractions favor this interpretation. Firstly, polysomal AFP is essentially nonreactive toward Con A–Sepharose, whereas rough microsomal AFP is maximally reactive (the 12% and 85% figures are respectively over- and underestimates because of residual contamination by extracellular protein (Peters et al., 1971) and cross-contamination among organelles; corrected values are $\simeq$3% and $\simeq$93%[3]). Clearly, these data point to the nascent AFP being released from the polysome as a nonglycosylated peptide, and receiving its complement, at least in part, of asparagine-linked oligosaccharide unit core residues *N*-acetylglucosamine and mannose when it reaches the rough endoplasmic reticulum (Schachter, 1974; Waechter & Lennarz, 1976; Bélanger et al., 1979). Secondly, from highest in the endoplasmic reticulum, the Con A reactivity decreases to a serum-type profile when the AFP molecules enter the Golgi apparatus. In the liver, the oligosaccharide unit peripheral residues sialic acid and galactose are specifically added in that organelle (Table I; Fleischer & Kervina, 1974; Schachter, 1974; Jamieson, 1977; Fleischer, 1978): the data are thus consistent with the addition of the peripheral residues galactose and sialic acid limiting the availability of the core residues *N*-acetylglucosamine and mannose for Con A binding. This changing pattern of Con A reactivity as the AFP molecules progress from the polysome through the secretory channels to the extracellular compartment and the observed distribution of organelle marker enzymes, similar to that in adult liver (Fleischer & Kervina, 1974), indicate in turn that the liver cell functional organization for processing of export glycoproteins is the same in the 12 day old rat as it is in the adult.

In adult rat liver, albumin is present in the rough microsomes as a larger precursor molecule (Edwards et al., 1976; Ikehara et al., 1976); under nondenaturing conditions, this proalbumin has a slower and more heterogeneous electrophoretic mobility than serum albumin (Peters, 1977). Thus, in all likelihood, the slow-migrating albumin recovered from the newborn rough microsomes also consists of precursor molecules. The slow mobility of microsomal AFP, however, is not necessarily indicative of a similar precursor because desialylation reduces the mobility of rat serum AFP (Ikehara et al., 1976), and, as discussed above, AFP in the endoplasmic reticulum is probably a nonsialylated product. The basis for the electrophoretic heterogeneity of rat AFP (Bélanger & Dufour, 1974; Watabe, 1974; Watanabe et al., 1975) is unclear at present. The presence of the "slow" and "fast" variants in rough microsomes indicates that they are not determined by the oligosaccharide unit peripheral residues galactose or sialic acid (added in the Golgi apparatus). This leaves open the possibility of a genetic control.

From present data and others in the literature, the scheme for the manufacturing and secretion of AFP in the liver now appears to be the following. AFP is synthesized and released from membrane-bound ribosomes as a nonglycosylated peptide. It becomes initially glycosylated as it moves into the rough endoplasmic reticulum, where it receives at least part of the core structure (*N*-acetylglucosamine and mannose) of its oligosaccharide unit(s); the 93% Con A reactivity suggests that most, if not all, AFP molecules are glycosylated, but no firm conclusion can be drawn at this time on whether all glycosylated molecules contain a single oligosaccharide unit. Whether all AFP molecules must pass through the smooth endoplasmic reticulum before reaching the Golgi apparatus is also uncertain at present (Peters et al., 1971). When the growing peptide enters the Golgi apparatus, it receives a variable number of peripheral carbohydrate residues (galactose and sialic acid) and apparently achieves a terminal lectin reactivity and microheterogeneity profile. The finished product is then transported through the plasma membrane compartment and released in the sinusoidal space. The entire process is completed in about 15 min, and the flow of AFP in the secretion path parallels that of albumin. Whether the scheme also includes the sequential processing of precursor molecules remains to be clarified.

Some additional conclusions can also be proposed in regard to albumin. The albumin content of the purified subcellular

[3] That is, 12% minus 9% contributed by the Con A reactive AFP in the contaminating nonpolysomal AFP (60% of 15%), and 85% + 8% to correct for the Con A nonreactive AFP contributed by the contaminating Golgi complex (2% contamination × tenfold higher AFP content per mg of protein × 37% Con A nonreactive AFP = 8).

fractions obtained here from newborn rat liver is remarkably similar to that found by Peters et al. (1971) in comparable fractions obtained from young adult rat liver. A related study by the same group (Peters & Peters, 1972) further indicated that under various physiological conditions the concentration of albumin in liver microsomes is directly proportional to its rate of synthesis, its rate of secretion remaining constant. Assuming that this is also valid for 12 day old rats, our data thus indicate that the albumin production rate per unit weight of liver is the same in 12 day old rats as it is in young animals; this conclusion is in agreement with other studies on albumin production by the rat liver during postnatal development (Yeoh & Morgan, 1974; Wise & Oliver, 1967). However, immunocytochemical studies have also indicated that the proportion of albumin-producing hepatocytes remains constant ($\simeq$15%) from 12 days after birth through adulthood (Guillouzo et al., 1976, 1978). Since the number of parenchymal cells per unit volume of liver decreases from 12 days through adulthood (Greengard et al., 1972), in order to maintain constant the albumin production rate per unit weight of liver from 12 days onward, its production rate per cell, therefore, must increase. With constant secretion rate and half-life (Peters & Peters, 1972; Yeoh & Morgan, 1974), it thus can be concluded that the rising level of plasma albumin after 10 days of age in rats (Yeoh & Morgan, 1974; Bélanger, 1975) only depends on the increases in the absolute number of albumin-synthesizing cells in the growing liver and in the albumin synthesis rate per producing hepatocytes. [Between 0 and 10 days, the pattern is the opposite: there is an increase in the rate of albumin synthesis per unit weight of liver but no increase in the production rate per cell (Yeoh & Morgan, 1974; Wise & Oliver, 1967). This reflects the changing proportion of liver cell populations: the hemopoietic cells disappear and the relative and absolute number of hepatocytes increases (Greengard et al., 1972).]

The data accumulated here support current concepts regarding the production, secretion, and processing of export peptides in the liver. They indicate that, in the rat, these subcellular functions develop into an adult-type pattern early in the postnatal development. They also provide further evidence that the microheterogeneity of rat AFP, as for a large number, if not all, secreted glycoprotein including AFP in other species, is at least in part due to a variable peripheral structure of its carbohydrate moiety (Kerckaert et al., 1977; Purves et al., 1970; Alpert et al., 1972; Zimmerman & Madappally, 1973, Lester et al., 1976). A number of carbohydrate-affecting or carbohydrate-dependent posttranslation or postsecretion mechanisms could account for the changing lectin-binding behavior of extracellular AFP observed during development or pathological states of the liver (Smith et al., 1977; Bélanger, 1975); differences in the glycosylating enzyme functions of AFP-producing cells are possibly the major factors (Zimmerman & Madappally, 1973).

Because of its low and heterogeneous carbohydrate content, and its easy preparation in large quantities, rat AFP constitutes an interesting product for structural analyses of peptide-linked oligosaccharide units. Its typical production, secretion, and processing in neonatal liver also makes it an excellent probe for comparative studies of liver functions at earlier developmental stages. The sequential glycosylation of AFP in subcellular compartments finally offers an opportunity to investigate its putative carbohydrate-dependent functional heterogeneity (Savu et al., 1977: Valette et al., 1976; Zimmerman et al., 1977).

Acknowledgments

We are grateful to Dr. C. J. Smith for her critical review of this paper.

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