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D Gitlin, M Boesman

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Research Article

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Serum α-Fetoprotein, Albumin, and γG-Globulin in the Human Conceptus *

DAVID GITLIN † AND MARY BOESMAN

(From the Department of Pediatrics, University of Pittsburgh School of Medicine and the Children's Hospital of Pittsburgh, Pittsburgh, Pa.)

The serum of the human fetus contains a protein that is not present in the sera of normal children or adults (1, 2). This protein has been observed in the serum of the conceptus as early as 10 to 14 weeks in the gestational period, and it has been reported that the serum concentration of the protein decreases as gestation continues (1–7). Most investigators have been unable to detect the protein in fetuses older than 4 to 6 months' gestation (1–6, 8) or in prematurely born infants (5), but others have reported its presence in cord serum and in the serum of the newborn during the first week of life (9, 10).

The electrophoretic mobility of this serum fetoprotein at pH 8.6 is that of an α-globulin, the protein migrating somewhat slower than serum albumin but faster than the serum α_1-globulins at this pH (1–10). As is well known, fetal calf serum also contains an α-globulin, the mucoprotein fetuin (11), which is not found in adult bovine serum. Calf fetuin, however, has a molecular weight of approximately 45,000 (12) and contains galactose, whereas human serum α-fetoprotein appears to have a molecular weight similar to that of serum albumin, 65,000, and is galactose free (2–4). Bodman (8) has suggested that serum α-fetoprotein present in the human fetus of less than 4 months' gestation is actually fetuin without galactose, and he has reported that after 4 months' gestation, the α-fetoprotein acquires galactose and can react with antisera prepared against calf fetuin. On the other hand, de Muralt and Roulet (5) were unable to obtain a precipitin reaction between human serum α-fetoprotein and antisera against calf fetuin, and Masopust and Kotál (9) were unable to demonstrate any precipitin reaction between calf fetuin and an antiserum against human serum α-fetoprotein.

Tatarinov (10) has reported the presence of an α-fetoprotein in the sera of women after spontaneous abortion, suggesting that under these circumstances at least, α-fetoprotein can traverse the placenta in the direction of fetus to mother. Bodman (8) has indicated that the serum of the pregnant woman contains fetuin, and he has concluded that the placenta is permeable to α-fetoprotein when the protein acquires galactose. Other investigators have been unable to detect serum α-fetoprotein, electrophoretically or immunochemically, in either normal or toxemic pregnant women (1, 2, 5, 6, 9).

In the present study, the serum concentration of α-fetoprotein in the human conceptus was reinvestigated and compared to the serum concentrations of albumin and γG-globulin.

Methods

Serum, amniotic fluid, and urine samples. Serum was obtained at the time of delivery from the embryos and fetuses listed in Table I; the source of the serum, whether from cord blood or from blood obtained by heel puncture immediately after delivery, the manner of delivery, and the method used to estimate the gestational age of each conceptus are also given in Table I. Before a blood sample was taken, amniotic fluid was collected in some instances (Table II) by aspiration with needle and syringe directly from the amniotic sac. Bladder urine was obtained from fetus C5-64 of Table I by needle aspiration after exposure of the bladder by dissection and from five additional fetuses (Table III) by freezing the entire fetus to − 10° C and then dissecting the bladder from the frozen urine contained within it.

Blood was collected from 12 premature infants of different postnatal ages by heel puncture. These children were delivered after the spontaneous onset of labor at 34 to 36 weeks' gestation, the gestational period being taken

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† Address requests for reprints to Dr. David Gitlin, Dept. of Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, Pa. 15213.

TABLE I

The gestational periods, weights, and manner of delivery of the embryos and fetuses included in Figures 3, 4, and 6, together with their serum concentrations of α-fetoprotein, albumin, and γG-globulin

Conceptus	Etimated gestation	Weight	Delivery	Origin of serum*	Serum α-feto-protein	Serum albumin	Serum γG-globulin
	weeks	*g*			*mg/100 ml*	*g/100 ml*	*g/100 ml*
5	6.6†	1	Cesarean	Cord	6.65	0.15	0.07
4	9.4‡	5	Cesarean	Cord	191	0.43	0.06
C3-65	9.5‡	6	Cesarean	Cord	196	0.44	0.10
C5-64	12.8‡	20	Cesarean	Cord	279	0.56	0.05
6	17.8‡	180	Cesarean	Cord	127	1.6	0.17
1	20.0‡	320	Cesarean	Cord	154	1.6	0.17
2	21.0‡	400	Cesarean	Cord	140	1.7	0.12
7	22.2‡	470	Cesarean	Cord	127	3.0	0.10
WH	26§	825	Spontaneous	Cord	11.9	4.0	
J3-65	26	975	Cesarean	Cord	54.7	3.4	1.2
TL	29	1,275	Incompetent cervix	Cord	44.4	2.8	
P12	30	1,370	Extraction-abruption	Heel	43.4	3.2	0.92
GR	31	1,600	Spontaneous	Cord	20.3	3.0	
PS	32	1,600	Spontaneous	Heel	12.1	2.5	0.86
P9	34	2,020	Spontaneous	Heel	1.60	2.4	1.38
HV	34	2,350	Spontaneous	Cord	1.00	2.1	
P3	35	2,250	Spontaneous	Heel	9.20	2.4	1.38
P5	35	2,270	Spontaneous	Heel	13.2	2.8	0.70
CC	35	2,466	Spontaneous	Cord	0.89	4.2	1.88
BU	35	2,740	Spontaneous	Cord	8.12	3.4	
AZ	35	2,990	Spontaneous	Cord	1.27	4.0	
P11	36	2,340	Spontaneous	Heel	17.8	3.6	1.10
P18	36	2,350	Spontaneous	Heel	16.5		1.40
SM	36	2,920	Cesarean	Cord	8.12	3.4	
PB1	36	3,290	Spontaneous	Heel	1.83	4.3	2.3
CE	37	2,360	Spontaneous	Cord	6.35	4.2	0.90
P1	37	2,580	Spontaneous	Heel	4.06	3.2	
GO	37	3,115	Cesarean	Cord	14.0	3.4	
UR	37	3,540	Cesarean	Cord	10.7	3.4	
MA	37	3,632	Cesarean	Cord	1.39	3.7	
CM	38	3,200	Spontaneous	Cord	1.27	3.2	1.34
10	38	3,250	Spontaneous	Cord	4.90	2.4	1.00
CY	38	3,290	Spontaneous	Cord	8.60	4.2	2.20
CN	38	3,374	Spontaneous	Cord	1.65	4.2	2.20
95-65	38	3,640	Spontaneous	Heel	3.99	4.2	1.14
PB4	39	3,374	Spontaneous	Cord	1.83	4.3	2.30

* Cord = blood from umbilical cord; heel = blood obtained by heel puncture.
† Based on Streeter's horizons (13); crown-rump length = 2.7 cm.
‡ Based on Patten's data for crown-rump length (14).
§ From 26 weeks on, the gestational period was taken to be 2 weeks less than the interval between the last menstrual period and the time of delivery.

as 2 weeks less than the interval between the mother's last menstrual period and the time of delivery; the infants weighed between 2.02 and 2.40 kg at birth. Blood was also obtained by venipuncture from four pregnant women during their second trimester and from the mothers of six of the infants in Table I at the time of delivery (Table V).

Zone electrophoresis. Beckman cellulose acetate strips (15) were used as the supporting medium for zone electrophoresis, which was performed in barbiturate buffer of ionic strength 0.075 at pH 8.6 with 35 v per cm length for 20 minutes; 0.25 to 1.0 μl of fluid was applied. After electrophoresis, the strips were stained with 2% Ponceau S in 30% trichloroacetic acid and 30% sulfosalicylic acid, washed in 5% acetic acid, dried at 100° C, and scanned photoelectrically with the Beckman Analytrol. Each fluid sample underwent electrophoresis on a minimum of three separate occasions; the results were almost identical from one occasion to the next.

Quantitative estimation of specific serum proteins. The concentrations of albumin, γG-globulin, and α-fetoprotein in the various fluids were determined immunochemically by the method of Mancini, Carbonara, and Heremans (16) with specific rabbit antisera and an agar concentration of 1.5 g per 100 ml of 0.1 M borate buffer at pH 8.6. Preparation of the rabbit antialbumin and anti-γG-globulin antisera was performed as described elsewhere (17). The antifetoprotein antisera were prepared by injecting rabbits subcutaneously once every 2 weeks for a total of eight injections with 0.03 ml of serum from either fetus 1 or fetus 2 of Table I emulsified in Freund's complete adjuvant. When used for the quantitative deter-

mination of α-fetoprotein, the antisera were adsorbed with pooled human serum from normal adult males; 0.2 ml of adult serum was required per ml of rabbit antiserum to inhibit completely or remove all antibodies capable of precipitating any of the proteins in adult serum.

Human serum albumin [1] and γG-globulin,[2] obtained by low temperature ethanol fractionation of pooled adult serum (18, 19), were employed for the standardization of the antisera against these proteins. The serum of fetus 6 (Table I) was used as the reference standard for α-fetoprotein: the concentration of α-fetoprotein in this serum was determined by planimetry of the scans obtained from electrophoresis of the serum on cellulose acetate; the concentration of albumin in this serum was determined immunochemically, and the concentration of α-fetoprotein was taken to be the area under the electrophoretogram representing α-fetoprotein divided by the area for albumin times the serum concentration of albumin. It may be noted that the concentrations of α-fetoprotein in the different fluid aliquots relative to each other are the same as the determined absolute concentrations are to each other regardless of an error in the absolute determination of α-fetoprotein in fetal serum 6 by this method.

The lowest concentrations of albumin, γG-globulin, and α-fetoprotein that could be determined with the antisera employed were 10 mg, 10 mg, and 0.025 mg per 100 ml of fluid, respectively. When the concentration of albumin or α-fetoprotein in a given fluid was too low to be detected, the fluid was dialyzed against water, lyophilized, and reconstituted with 0.15 M NaCl to a smaller volume than the original volume; in this manner, the maternal sera were concentrated fivefold and amniotic fluids were concentrated ten- to twentyfold.

Rabbit antiserum specific for human transferrin was also prepared and was employed with the method of Mancini and associates to quantitate this protein in chromatographic and ultracentrifugal fractions of fetal serum; the purified human transferrin used and the method of preparing the antiserum are described elsewhere (17, 20).

Qualitative immunochemical methods. Immunoelectrophoresis was performed according to the method of Scheidegger (21) with an agar concentration of 1.5 g per 100 ml of 0.1 M borate buffer at pH 8.6. The same agar solution was used for the preparation of micro-Ouchterlony plates (22, 23). In addition to the antisera just described, rabbit antiserum specific for human serum orosomucoid and guinea pig antiserum against human growth hormone were also prepared; the antigens used and the methods of preparing the antisera are described elsewhere (17, 24). Other materials included purified human placental lactogen-growth hormone and a rabbit antiserum to this protein.[3] Each fluid was examined by immunoelectrophoresis on a minimum of three separate occasions with identical observations being obtained for the given fluid in each instance.

Fractionation of fetal serum. To obtain an estimate of the molecular size of α-fetoprotein, we passed 5 ml of cord serum from infant UR through a 2.5-cm by 100-cm column of Sephadex G-200 on two separate occasions and collected the effluent in 5-ml aliquots in each instance, using 0.1 M NaCl for elution; the concentrations of albumin, γG-globulin, transferrin, and α-fetoprotein in each aliquot were estimated semiquantitatively in micro-Ouchterlony plates and graded from 0 to 4+ on the basis of the intensity of the precipitin lines produced between the serum proteins and the specific antisera. The qualitative results thus obtained were the same for the effluents of both columns. An estimate of the molecular size of α-fetoprotein was also made with the method of Martin and Ames (25) by ultracentrifugation in a sucrose gradient: 0.4 ml of serum from fetus 6, to which was added 0.1 ml of a 5 g per 100 ml solution of γG-globulin (Squibb 357-1), was dialyzed against 5 g per 100 ml sucrose in 0.1 M NaCl and placed on top of a sucrose gradient prepared as described by Kunkel, Rockey, and Tomasi (26), the sucrose gradient being from 5 g per 100 ml at the top of the 1-cm by 5-cm tube to 20 g per 100 ml at the bottom. Two tubes prepared in this manner were centrifuged for 18 hours at 110,000 times *g* in a swinging bucket head of the Spinco model L ultracentrifuge; the bottom of each tube was then pierced with a no. 25 needle, and the contents were collected in 2.3-ml aliquots, which were assayed for γG-globulin, transferrin, α-fetoprotein, albumin, and orosomucoid.

Results

With cellulose acetate electrophoresis, the presence of an α-fetoprotein migrating between albumin and the α_1-globulins (Figure 1A) could be detected in the sera of all of the embryos and fetuses delivered between 9.4 and 30 weeks' gestation with the exception of the fetus delivered at 26 weeks after spontaneous labor. The protein could not be detected in the serum of the 6.6-week embryo and was not observed in the sera of those infants delivered after 31 weeks' or more gestation or in the sera of adults (Figure 1).

Four different rabbit antisera prepared against fetal serum and then adsorbed with pooled normal adult serum each gave but a single precipitation line when reacted against fetal serum either on immunoelectrophoresis (Figure 2) or in Ouchterlony plates. The fetoprotein precipitated by the adsorbed antisera had a mobility slightly slower than that of albumin (Figure 2), corresponding in relative mobility to the α-fetoprotein observed by zone electrophoresis on cellulose acetate. With

[1] No. 1755D, Merck Sharp and Dohme, Philadelphia, Pa.

[2] No. 357-1, Squibb, New York, N. Y.

[3] Kindly supplied by Dr. Melvin M. Grumbach of the University of California Medical School at San Francisco.

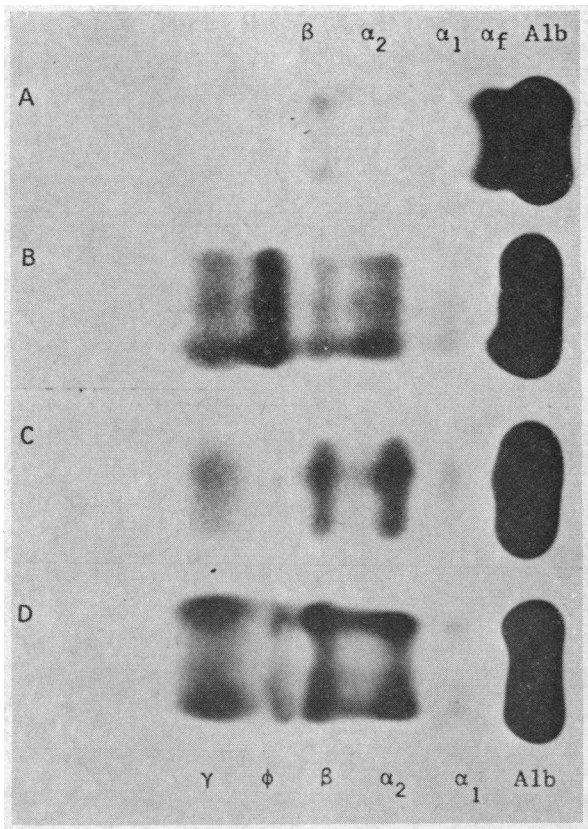

FIG. 1. ELECTROPHORESIS ON CELLULOSE ACETATE. A: Serum from C5-64, 12.8 weeks' gestation. B: Plasma from infant PS, 32 weeks' gestation. C: Serum CY, 38 weeks' gestation. D: Serum from mother AX, 12 weeks' gestation. Symbol α_f indicates α-fetoprotein; φ indicates area of origin; alb is albumin.

the adsorbed antisera in Ouchterlony plates, α-fetoprotein could be detected in the sera of all of the embryos and fetuses of Table I and in the sera of infants during the newborn period. In no instance did the adsorbed antisera precipitate any of the serum proteins of normal adults (Figure 2). Although the electrophoretic mobility of albumin B in individuals with paralbuminemia is similar to that of α-fetoprotein (27), and it has been suggested that albumin B and α-fetoprotein may be the same protein (7), there was no detectable precipitation reaction between the adsorbed antisera and albumin B (Figure 2).

The antisera against human placental lactogen-growth hormone and human pituitary growth hormone failed to precipitate α-fetoprotein either in Ouchterlony plates or on immunoelectrophoresis, although precipitins in these antisera for their respective antigens were readily demonstrated by these techniques. In accord with these findings, the antisera specific for α-fetoprotein failed to precipitate either of the two hormones on immunoelectrophoresis or in Ouchterlony plates.

Upon filtration of serum UR through Sephadex G-200, the peak concentration of α-fetoprotein in the effluent appeared in the same area as the peak concentrations of transferrin and albumin, suggesting that the molecular size of α-fetoprotein is in the range of the molecular sizes of the latter two proteins. In accord with this observa-

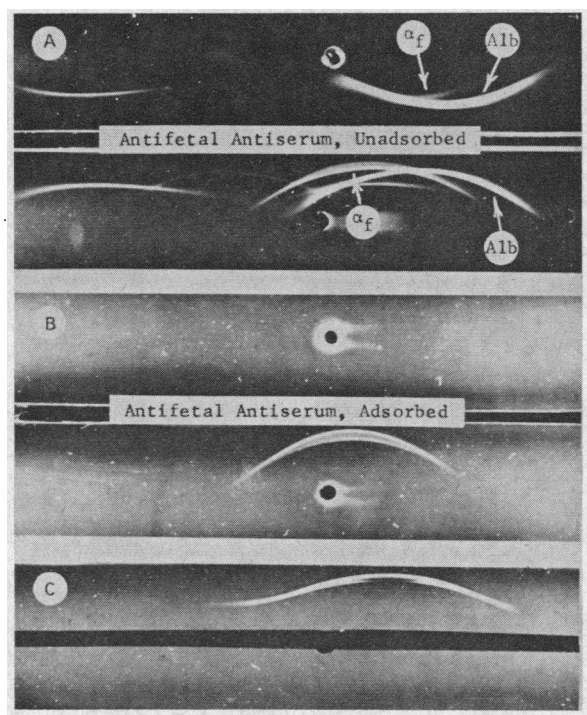

FIG. 2. IMMUNOELECTROPHORESIS; ANODE TO THE RIGHT. A: Unadsorbed antiserum against fetal serum proteins in middle trough diffusing against serum 5, 6.6 weeks' gestation, in upper well and serum 4, 9.4 weeks' gestation, in lower well after electrophoresis. Arrow α_f indicates precipitation of α-fetoprotein and arrow alb indicates the precipitin band for albumin. B: Central trough contains antifetal antiserum adsorbed with pooled normal adult serum; fetal serum 4 underwent electrophoresis in lower well and normal adult serum in upper well. C: Electrophoresis of serum from a patient with albumin B paralbuminemia; after electrophoresis, the block was cut into upper and lower halves. Antialbumin antiserum was then allowed to diffuse into the block from upper side of figure, and adsorbed antifetal antiserum was allowed to diffuse into the block from the lower side of the figure; the antifetal antiserum did not precipitate albumin B.

tion, ultracentrifugal fractionation of fetal serum 6 in sucrose gradient tubes resulted in the appearance of the peak concentration of α-fetoprotein in the same area as that of albumin and transferrin. With 4.6 S as the sedimentation coefficient, $s_{w,20}$, for serum albumin (28), the calculated sedimentation coefficient (25) for α-fetoprotein in each tube uncorrected for protein concentration was 5.0 S; as an indication of the linearity of the sucrose gradient, the calculated sedimentation coefficients for γG-globulin and transferrin in the same tubes were 6.9 ± 0.1 S and 5.6 S ± 0.1 S, respectively, values that agree reasonably well with the coefficients of approximately 7 S and 5.5 S usually accepted for these proteins (29).

Changes in the serum concentrations of α-fetoprotein, albumin, and γG-globulin with gestational age. The serum concentrations of α-fetoprotein, albumin, and γG-globulin at the time of delivery in the embryos and fetuses studied are listed in Table I; all determinations were made immunochemically. The serum concentration of α-fetoprotein was relatively low in the 6.6-week embryo, but it rose rapidly to reach a maximum at approximately 13 weeks' gestation; the concentration of α-fetoprotein then declined so rapidly (Figure 3) that the average concentration between approximately 18 and 22 weeks' gestation was only 3.2% of that at maximum, and between 34 and 39 weeks it was only 1.6% of maximum. If the data are replotted semilogarithmically (Figure 4), it will be noted that the α-fetoprotein concentration in those infants delivered without preceding labor declined almost as a single exponential until approximately 34 weeks' gestation. Of three infants born between 26 and 32 weeks' gestation after the spontaneous onset of labor, the α-fetoprotein concentration in at least one, WH, delivered at 26 weeks, was below the general trend; due to the lack of data for infants delivered between 30 and 36 weeks' gestation

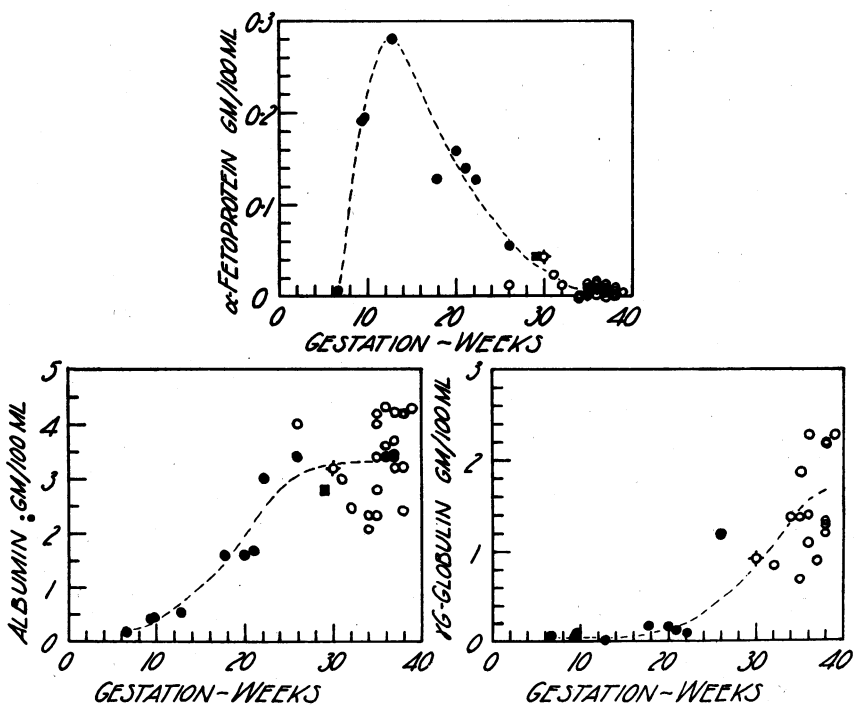

FIG. 3. SERUM CONCENTRATIONS OF ALBUMIN, α-FETOPROTEIN, AND γG-GLOBULIN IN THE CONCEPTUSES OF TABLE I PLOTTED LINEARLY. In this Figure and in Figures 4 and 7, the broken lines are intended only to indicate a possible trend; for albumin and α-fetoprotein, the trend indicated is for infants delivered without preceding labor. Manner of delivery: solid circles = cesarean section; solid square = incompetent cervix; hollow crossed circle = extraction for abruption of placenta; hollow circles = spontaneous.

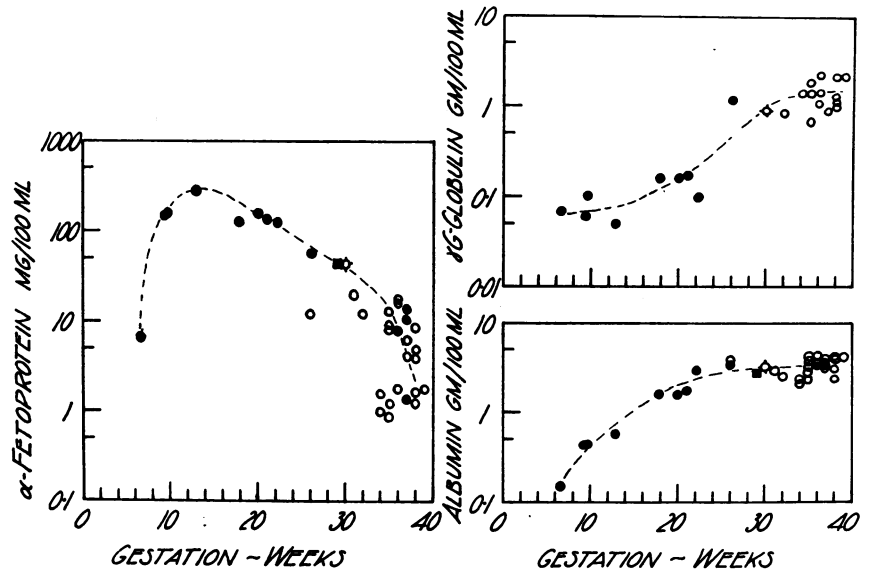

FIG. 4. SAME DATA AND SYMBOLS AS IN FIGURE 3 PLOTTED SEMILOGARITHMICALLY.

without labor, the data for the remaining two infants could not be evaluated. There was a relatively wide spread in α-fetoprotein concentration among infants born after 34 or more weeks' gestation (Figure 4), suggesting an increase in the rate of fall of α-fetoprotein in many of the infants beginning at some point after 30 weeks' gestation. The serum α-fetoprotein levels found in 12 infants of various postnatal ages clearly indicated a more rapid fall (Figure 5) in α-fetoprotein in the postnatal period than was present during the fetal period from 13 to 34 weeks' gestation; although these 12 infants were selected at random and all were born spontaneously, consecutive determinations of α-fetoprotein in five infants delivered with or without prior labor (Figure 6) showed a similarly rapid postnatal disappearance of α-fetoprotein. The average half-life of α-fetoprotein during the first week of the postnatal period for the five infants represented in Figure 6 was 5.1 days, and the average half-life after 7 days of postnatal life in four of the infants in whom it was measured was 3.1 days. The trend to a similar increase in the serum disappearance of α-fetoprotein soon after birth was also seen in the data from the 12 infants selected at random (Figure 5). The relative effect of hemoconcentration due to dehydration during the first week of life on α-fetoprotein disappearance was not ascertained; serum albumin concentrations in the five infants of Figure 6, however, did not vary more than 10% from the level present at birth during the period of study.

The serum albumin concentration in the 6.6-week embryo was only 0.15 g per 100 ml. The albumin level increased steadily until approximately 26 weeks' gestation, by which time concentrations similar to those seen in normal adults were attained; after 26 weeks' gestation the average albumin concentration remained relatively constant (Figures 3 and 4).

Between 6.6 and approximately 22 weeks' gestation, the serum concentration of γG-globulin was less than 0.2 g per 100 ml; the average concentration of γG-globulin between 34 and 39 weeks' gestation was approximately 1.5 g per 100 ml. Thus, most of the increase in serum γG-globulin concentration in the fetus occurred between 22 and 26 weeks' gestation (Figures 3 and 4).

The average blood and plasma volumes per kilogram of body weight in premature infants born after 26 to 35 weeks' gestation have been reported to be similar to those measured in full-term infants (30, 31); therefore, during the gestational period from 26 weeks to term, at least, the plasma and blood volumes appear to be approximately proportional to body weight. Data for plasma and blood volumes on fetuses of less than 25 weeks' gestation are lacking. To

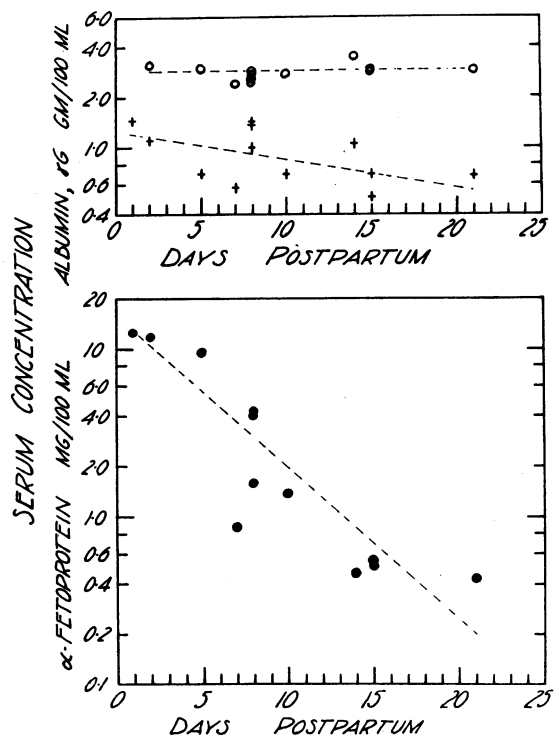

FIG. 5. SERUM CONCENTRATIONS OF α-FETOPROTEIN (SOLID CIRCLES), ALBUMIN (HOLLOW CIRCLES), AND γG-GLOBULIN (CROSSES) IN 12 INFANTS OF VARIOUS POSTNATAL AGES. The infants were between 34 and 36 weeks' gestation at birth and weighed between 2.02 and 2.40 kg. The broken lines represent hypothetical serum disappearance curves drawn through the actual values obtained: the broken line through the α-fetoprotein data represents a hypothetical half-life of 3.5 days, that through the γG-globulin data represents a half-life of 25 days, and that through the albumin values has a half-life of infinity.

obtain a crude approximation of the relative change in the amount of α-fetoprotein in the fetal circulation, exclusive of the placenta and umbilical cord, we multiplied the serum concentration of α-fetoprotein in each conceptus by the body weight of the conceptus. If it can be assumed that the plasma volume is proportional to body weight, the calculated values would be related to each other approximately as were the amounts of α-fetoprotein in the fetal circulation exclusive of that in the cord and placenta. The results of these calculations are shown in Figure 7; as already indicated, the results simply indicate a trend in the change of circulating α-fetoprotein with gestational age. It may be noted, however, that relatively large differences in the plasma volume per unit weight of conceptus before 18 weeks' gestation would not significantly alter the trend indicated in Figure 7 during this period, due to the relatively low weight of the conceptuses involved. It would appear from Figure 7 that the amount of α-fetoprotein in the fetal circulation may reach a maximum not at 12 to 13 weeks, the time of maximal serum concentration, but rather at approximately 22 to 23 weeks' gestation. Between approximately 22 and 30 to 32 weeks' gestation, the amount of circulating α-fetoprotein would appear to remain relatively constant despite fetal growth with the exception of fetus WH. At 30 or more weeks' gestation, the amount of α-fetoprotein in the circulation seemed to decrease dramatically, a reflection of the rapid fall in serum concentration after 30 weeks' gestation that was noted in Figure 4.

The marked rise in the apparent amount of circulating albumin and γG-globulin after 22 to 23 weeks' gestation (Figure 7) is in sharp contrast to the changes in the apparent amount of circulating α-fetoprotein during the same period.

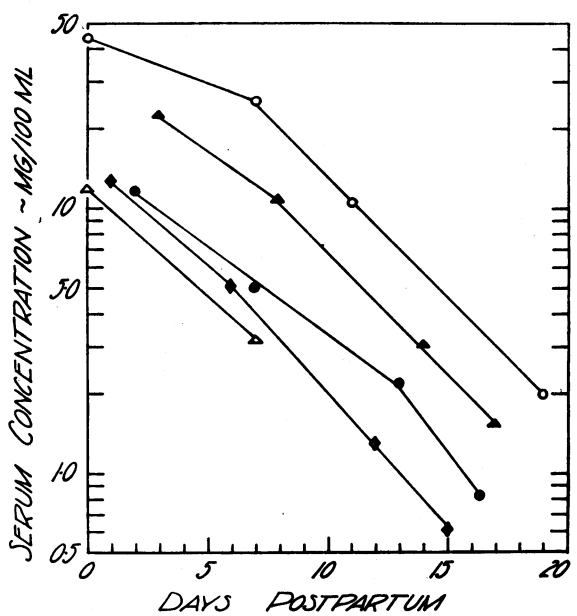

FIG. 6. THE DISAPPEARANCE OF α-FETOPROTEIN IN FIVE INFANTS DURING THE IMMEDIATE POSTNATAL PERIOD. Hollow triangles indicate spontaneous delivery (WH) at 26 weeks' gestation; hollow circles, delivery caused by incompetent cervix (TL) at 29 weeks; solid triangles, cesarean section at 30 weeks; solid circles, spontaneous delivery at 36 weeks; solid diamonds, spontaneous delivery at 36 weeks.

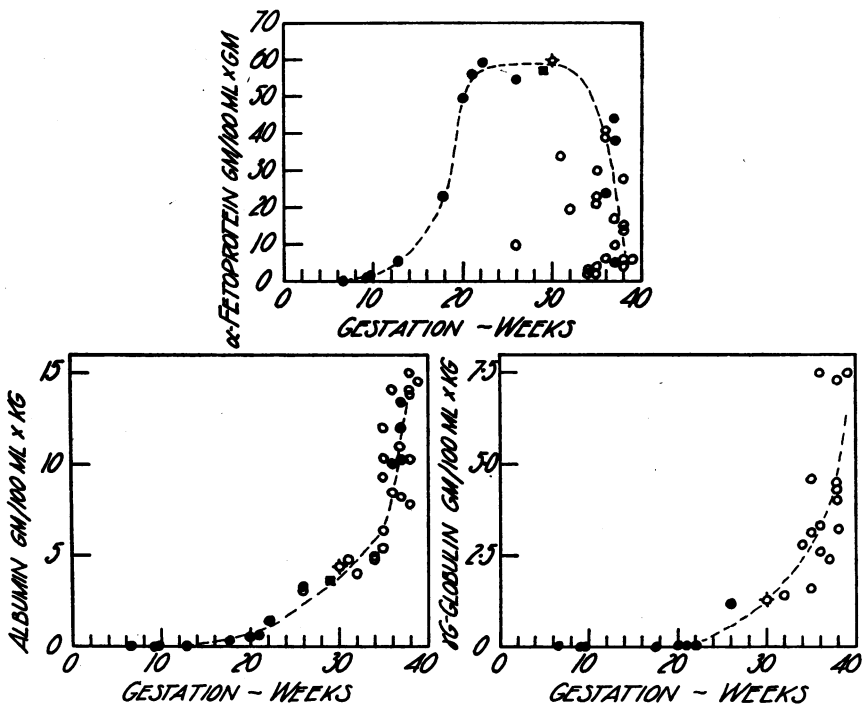

FIG. 7. THE SERUM CONCENTRATIONS OF α-FETOPROTEIN, ALBUMIN, AND γG-GLOBULIN MULTIPLIED BY THE BODY WEIGHT OF THE CONCEPTUS. The symbols and the significance of the broken lines are as in Figure 3.

Amniotic fluid and bladder urine analyses. The concentrations of α-fetoprotein and albumin in the five amniotic fluids studied are given in Table II. In the 6.6-week embryo, the concentration of α-fetoprotein in amniotic fluid was 2.9% of that in the serum, and from 9.4 to 12.8 weeks' gestation it varied from 0.16 to 0.55% of that in serum; at 39 weeks, the amniotic fluid α-fetoprotein concentration was below 1.4% of the serum α-fetoprotein. The concentration of amniotic fluid albumin, on the other hand, varied from 3.0 to 6.4% of the serum albumin concentration between 6.6 weeks' and 39 weeks' gestation. Thus, the concentration of albumin in amniotic fluid relative to the albumin concentration in serum was greater than the same ratio for α-fetoprotein.

The concentrations of albumin and α-fetoprotein in the six urine samples studied are shown in Table III. Unfortunately, both serum and amniotic fluid were obtained from only one of these fetuses, C5-64.

TABLE II

Concentrations of α-fetoprotein and albumin in amniotic fluid

Conceptus	Gestation	Amniotic fluid		Amniotic fluid:serum	
		α-Fetoprotein	Albumin	α-Fetoprotein	Albumin
	weeks	*mg/100 ml*	*mg/100 ml*		
5	6.6	0.152	6.0	0.029	0.040
4	9.4	0.61	23	0.0032	0.054
C3-65	9.5	1.08	13	0.0055	0.030
C5	12.8	0.45	36	0.0016	0.064
PB4	39	<0.025*	150		0.035

* Limit of sensitivity = 0.025 mg per 100 ml fluid.

TABLE III

Concentrations of α-fetoprotein and albumin in bladder urine

Conceptus	Gestation*	α-Fetoprotein	Albumin
	weeks	*mg/100 ml*	*g/100 ml*
C1-64	11.0	5.34	13
C8-64	11.0	11.9	30
C4-64	12.4	0.45	<2†
C6-64	12.4	2.95	10
C5-64	12.8	0.45	<2†
C7-64	13.2	5.21	23

* Based on Patten's data (14).

† Below limits of sensitivity, which was 2 mg per 100 ml of original urine.

TABLE IV

Ratios of albumin to α-fetoprotein concentrations in serum, amniotic fluid, and urine

Conceptus	Gestation	Serum	Amniotic fluid	Urine
	weeks			
5	6.6	22.6	39.5	
4	9.4	2.25	37.7	
C3-65	9.5	2.24	12.0	
C1-64	11.0			2.4
C8-64	11.0			2.5
C4-64	12.4			<5
C6-64	12.4			3.3
C5-64	12.8	2.01	80.0	<5
C7-64	13.2			4.4

It will be noted from Table IV that between 9.4 and 12.8 weeks' gestation, the ratio of albumin to α-fetoprotein in the serum varied only from 2.01 to 2.25; in the same three conceptuses, the albumin to α-fetoprotein ratios in amniotic fluid were from 12 to 80, or approximately 5 to 40 times the serum ratios. On the other hand, in four conceptuses from 11.0 to 13.2 weeks' gestation, the ratios of albumin to α-fetoprotein in the urine were from 2.4 to 4.4, or approximately only 1 to 2 times the serum ratios of the 9.4- to 12.8-week conceptuses and only one-half to one-sixteenth of the amniotic fluid ratios of the latter.

Maternal serum concentrations of α-fetoprotein, albumin, and γG-globulin. Immunochemical analysis of the sera of ten pregnant women failed to reveal detectable amounts of α-fetoprotein (Table V); the gestational period represented by these women was from 12 to 38 weeks. Serum was obtained from both mother and infant in six instances between 26 and 38 weeks' gestation; the fetal albumin concentration was higher than the respective maternal albumin concentration in five of these paired sera, and the fetal γG-globulin concentration was higher than the maternal γG-globulin concentration in three.

Discussion

If the relative rate of α-fetoprotein catabolism and the intravascular-extravascular distribution of α-fetoprotein are assumed to be approximately constant in the conceptus, particularly after 18 weeks' gestation, then the relative rates of synthesis can be estimated. Although the maximal serum concentration of α-fetoprotein occurred at approximately 13 weeks in the conceptuses studied in this report, the relative amount of circulating α-fetoprotein based on the weight of the conceptus appeared to increase until approximately 22 weeks' gestation, suggesting that there was a continued increase in the total amount of α-fetoprotein synthesized during the 13- to 22-week period. Between 13 and 22 weeks, however, there was a marked decrease in serum α-fetoprotein concentration; this fall would seem to be attributable to an increase in the rate of growth in the conceptus that exceeded the increase in the amount of α-fetoprotein synthesized during this period. Between 22 and 30 to 32 weeks' gestation, the calculated relative amount of circulating α-fetoprotein in the fetus remained relatively steady; the data suggest that the total

TABLE V

Maternal serum concentrations of α-fetoprotein, albumin, and γG-globulin

Patient	Gestation	α-Fetoprotein		Albumin		γG-Globulin	
		Mother	Fetus	Mother	Fetus	Mother	Fetus
	weeks	*mg/100 ml*	*mg/100 ml*	*g/100 ml*	*g/100 ml*	*g/100 ml*	*g/100 ml*
AX	12*	<0.005		4.0		2.2	
DX	23*	<0.005		3.2		1.6	
WX	23*	<0.005					
YX	24*	<0.005		3.2		2.2	
M-J3-65	26†	<0.005	54.7	3.0	3.4	1.6	1.2
M-CC	35‡	<0.005	0.89	3.6	4.2	1.6	1.9
M-CE	37‡	<0.005	6.35	3.2	4.2	0.92	0.90
M-CN	38‡	<0.005	1.65	3.2	4.2	1.6	2.2
M-CM	38‡	<0.005	1.27	3.6	3.2	1.6	1.3
M-CY	38‡	<0.005	8.60	3.2	4.2	1.6	2.2

* Pregnancy not interrupted.
† Therapeutic interruption.
‡ Spontaneous delivery.

rate of synthesis of α-fetoprotein reached a plateau at approximately 22 weeks' gestation and that this plateau was maintained for approximately 8 to 10 weeks despite continued fetal growth during this period. It is to be emphasized, as has been pointed out earlier in this report, that the relative data on circulating α-fetoprotein are only approximations based on the assumption that the plasma volume of the conceptus is approximately proportional to body weight.

After 30 to 32 weeks' gestation, the rate of fall in α-fetoprotein concentration increased, and the estimated relative amount of circulating α-fetoprotein fell rapidly as well; both events suggest a relatively sudden decrease in the rate of α-fetoprotein synthesis. The range of serum α-fetoprotein concentrations in infants born after 32 weeks' gestation was from 0.89 to 17.8 mg per 100 ml; since three infants born of cesarean section at 37 weeks without labor had serum values with a range of 1.39 to 14.0 mg per 100 ml, it would seem unlikely that this decrease in α-fetoprotein synthesis was related to labor as cause and effect. Because of the wide range of α-fetoprotein concentrations in the infants born during this period, the rate of fall of α-fetoprotein could not be determined. During the immediate postnatal period, α-fetoprotein disappeared from the serum with an average half-life of approximately 5 days, and after the first week, it had an average half-life of approximately 3 days. Since the rapid postnatal fall in α-fetoprotein was evident in infants born with or without prior labor, the data indicate that α-fetoprotein synthesis was curtailed at or before birth. At least one premature infant delivered after spontaneous labor, WH, had a low serum α-fetoprotein concentration as well as a relatively low calculated amount of circulating α-fetoprotein, suggesting that synthesis of α-fetoprotein had also been curtailed prematurely in this instance, perhaps 1 to 2 weeks before the onset of labor based on the trend seen in the fetuses between 30 and 34 weeks' gestation. Since there was a lack of data on infants born without labor after 30 weeks' gestation, the status of such infants as GR, PS, P9, and HV as regards premature curtailment of synthesis could not be evaluated.

It is almost unnecessary to state that the half-life of the serum disappearance of α-fetoprotein as used in this report should not be construed to mean the disappearance of α-fetoprotein due to degradation, since it is possible that some synthesis of α-fetoprotein persisted during the early neonatal period in some infants. However, in view of the observation that the postnatal fall after 1 week appeared to be exponential, it would seem that synthesis after 7 days of postnatal life was either negligible or decreased exponentially as well.

Bergstrand and Czar obtained an electrophoretic fraction of fetal serum that contained both α-fetoprotein and albumin; ultracentrifugal analysis of this fraction revealed only a single peak with slight asymmetry, and these authors concluded that the molecular weight of α-fetoprotein is similar to that of albumin (2). In the present study, we found that the results of gel filtration of α-fetoprotein through Sephadex G-200 are compatible with a molecular size for α-fetoprotein between those of albumin, molecular weight 65,000, and transferrin, molecular weight 90,000 (32). In accord with this observation, ultracentrifugal analyses indicated that the sedimentation coefficient of α-fetoprotein, $s_{w,20}$, was approximately 5.0 S.

The ratio of albumin to α-fetoprotein was estimated in the amniotic fluid or urine of several conceptuses between 11 and 13.2 weeks' gestation. The data suffer from the disadvantage that, but for one instance, the amniotic fluids were obtained from different conceptuses than were the bladder urine samples. Nevertheless, the albumin: α-fetoprotein ratios in amniotic fluid were much higher than the same ratios in the bladder urine of fetuses of similar gestational age, indicating either that the clearance of α-fetoprotein from amniotic fluid at this stage of gestation is greater than that of albumin, or that the clearance of abumin into amniotic fluid by routes other than via the urine is greater than that of α-fetoprotein. Neither albumin nor α-fetoprotein is significantly decreased in amniotic fluid by maintenance of the fluid at 4° C for months, suggesting that differential destruction of α-fetoprotein by proteolytic enzymes within the fluid might not account for the ratio differences between amniotic fluid and urine. It is possible that the amniotic fluid ratio could be explained on the basis of protein transfer between the fetus and amniotic fluid across

such areas as the fetal lungs or skin; in this case, the area of transfer must have a greater selective permeability to the two proteins than was observed for the urinary tract, or else the kidney must reabsorb α-fetoprotein more effectively than albumin, providing the kidney tubules reabsorb either. On the other hand, it has been shown that when the pregnant woman is given labeled albumin intravenously, albumin in amniotic fluid may attain a higher specific activity than in fetal serum (33, 34); this is in sharp contrast to the behavior of labeled γG-globulin and other plasma proteins, which maintain a higher specific activity in fetal serum than in amniotic fluid. Thus, another possible explanation for the observed amniotic fluid albumin: α-fetoprotein ratio may be passage of small amounts of albumin from the mother into the amniotic sac without first passing through the fetus.

In all instances, the albumin: α-fetoprotein ratio in bladder urine was somewhat higher than the same ratio in the sera of comparable conceptuses. The urinary clearance of albumin, therefore, seemed to be higher than the clearance of α-fetoprotein at this stage of development.

In accord with the findings of others (1, 2, 5, 6, 9) α-fetoprotein could not be detected in the serum of the normal pregnant woman, the limit of sensitivity of the technique used in this study permitting the detection of 0.005 mg α-fetoprotein per 100 ml serum. The relation of the α-fetoprotein detected by Tatarinov (10) in the sera of women after spontaneous abortion and the relation of maternal fetuin detected by Bodman to the α-fetoprotein studied here remain to be established. Tatarinov reported that there are several immunologically distinct α- and β-globulins present in fetal serum that are not present in adult serum. One of the α-globulins described by Tatarinov is undoubtedly identical to the α-fetoprotein reported here, but with none of the adsorbed antisera were any of the other fetus-specific proteins found by Tatarinov detected in this study. At least one of the fetal β-globulins described by Tatarinov could be identified with the present antisera when the antisera were only partially adsorbed with adult serum, but this β-globulin was not specific for the fetus, since antibodies against it could be bound by the addition of more adult serum. That other fetus-specific serum proteins besides α-fetoprotein may be present in fetal serum cannot be denied, and the differences between the observations of Tatarinov and our own may be attributable to differences in the antisera used.

Although α-fetoprotein could not be found in the sera of normal pregnant women, it should be pointed out that this observation does not preclude transfer of small amounts of α-fetoprotein across the placenta, since the maternal volume of distribution is vastly greater than that of the fetus. Nevertheless, the maximal amount of α-fetoprotein that could be transferred would be less than 0.005 mg times 0.693 times the weight of the mother in kilograms divided by the half-life of the protein in days (35), or a maximum of approximately 0.06 mg per day in a woman of 60 kg with a half-life for α-fetoprotein of 3.5 days.

Since a relatively abrupt curtailment of α-fetoprotein synthesis seems to occur either at birth or during the few weeks before birth whether the infant is full-term or is born prematurely, it is tempting to speculate that the placenta may be the site of synthesis of this protein. Although it has been observed that the fetal opossum in the marsupial pouch continues to synthesize an analogue of serum α-fetoprotein in the absence of a placenta (36), it is possible that there may be more than one site of synthesis of serum α-fetoprotein.

Summary

The serum concentrations of α-fetoprotein, albumin, and γG-globulin in the human conceptus were determined immunochemically over the gestational period from 6.6 to 39 weeks and in the newborn during the first 3 weeks of life. It was found that the serum concentration of α-fetoprotein increased from 6.6 weeks to reach a maximal concentration at approximately 13 weeks; the concentration then decreased rapidly to reach levels of less than 2% of the maximum by 34 weeks' gestation. The newborn at term had detectable α-fetoprotein that disappeared from the serum with an average half-life of 5 days during the first week and with an average half-life of 3 days after the first week. The data suggest a sharp curtailment of α-fetoprotein synthesis either at birth or during the few weeks before birth whether the infant is full-term or is born prematurely.

Serum albumin concentrations in the conceptus

reached a plateau by 22 to 24 weeks' gestation and remained at these levels through the neonatal period; the average concentration of albumin in the neonatal period was slightly higher than that in the mothers studied. The serum concentration of γG-globulin between 6.6 and 22 weeks' gestation was less than 0.2 g per 100 ml. After 22 weeks the concentration of γG increased to reach levels seen in term infants by 26 weeks' gestation.

The albumin : α-fetoprotein ratios in amniotic fluid were much higher than those in bladder urine between 11 and 13.2 weeks' gestation, indicating the possible existence of an additional source of amniotic fluid albumin besides the urine. The clearance of albumin into the urine was greater than that of α-fetoprotein; the molecular size of α-fetoprotein was found to be similar to that of albumin by means of gel filtration and ultracentrifugation.

The serum of the pregnant women did not contain detectable amounts of α-fetoprotein.

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