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Development of γG, γA, γM, β_{1C}/β_{1A}, C′1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, α_1-antitrypsin, orosomucoid, β-lipoprotein, α_2-macroglobulin, and prealbumin in the human conceptus

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Research Article

The synthesis of γG, γA, γM, β_{1C}/β_{1A}, C′1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, α_1-antitrypsin, orosomucoid, β-lipoprotein, α_2-macroglobulin, and prealbumin was studied in 15 normal human embryos and fetuses of 29 days to 18 wk gestation and in the yolk sacs of four embryos from 5.5 to 11.5 wk gestation using tissue culture in ^{14}C-labeled amino acids followed by radioimmunoelectrophoresis. The human embryo as early as 29 day gestation synthesized β_{1C}/β_{1A}, C′1 esterase inhibitor, transferrin, hemopexin, α_1-antitrypsin, β-lipoprotein, α_2-macroglobulin, and prealbumin in culture. At 32 days gestation ceruloplasmin and orosomucoid were also synthesized, but synthesis of fibrinogen was not observed before 5.5 wk. Synthesis of γM occurred as early as 10.5 wk gestation, and γG synthesis was found in cultures as early as 12 wk gestation; γA synthesis was not detected in any of the tissue cultures. With the exception of the γ-globulins, each of the proteins studied was synthesized by the liver, but additional sites of synthesis for some of these proteins were also found. Synthesis of γG and γM occurred primarily in the spleen, but other sites of synthesis were noted as well.

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ABSTRACT The synthesis of γG, γA, γM, β_{1C}/β_{1A}, C'1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, α_1-antitrypsin, orosomucoid, β-lipoprotein, α_2-macroglobulin, and prealbumin was studied in 15 normal human embryos and fetuses of 29 days to 18 wk gestation and in the yolk sacs of four embryos from 5.5 to 11.5 wk gestation using tissue culture in ^{14}C-labeled amino acids followed by radioimmunoelectrophoresis. The human embryo as early as 29 day gestation synthesized β_{1C}/β_{1A}, C'1 esterase inhibitor, transferrin, hemopexin, α_1-antitrypsin, β-lipoprotein, α_2-macroglobulin, and prealbumin in culture. At 32 days gestation ceruloplasmin and orosomucoid were also synthesized, but synthesis of fibrinogen was not observed before 5.5 wk. Synthesis of γM occurred as early as 10.5 wk gestation, and γG synthesis was found in cultures as early as 12 wk gestation; γA synthesis was not detected in any of the tissue cultures. With the exception of the γ-globulins, each of the proteins studied was synthesized by the liver, but additional sites of synthesis for some of these proteins were also found. Synthesis of γG and γM occurred primarily in the spleen, but other sites of synthesis were noted as well.

Changes in the concentrations of most of these proteins and plasminogen in embryonic and fetal serum from 5.5 to 41 wk gestation, in amniotic fluid from 6.5 to 38 wk gestation, and in the sera of neonates during the 1st 3 wk postpartum are described. Although γA, γM, ceruloplasmin, or haptoglobin were not detectable in some of the embryonic and fetal sera, γA and ceruloplasmin were both present as early as 6.5 wk gestation, haptoglobin by 9.5 wk gestation, and γM by 17 wk gestation. Each of the other proteins were present in all of the sera examined.

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INTRODUCTION

In an extensive immunoelectrophoretic study of human embryonic and fetal sera, Scheidegger, Martin, and Riotton demonstrated the presence of prealbumin, albumin, an α_1-globulin, an α_2-globulin, and a β-globulin, which presumably was transferrin, as early as 8 wk of gestation (1); seven additional proteins were detected, including γG, beginning at 12–14 wk gestation (1). The different proteins, however, were identified only by their relative positions in the immunoelectrophoretic pattern, and, with but few exceptions, have been almost impossible to relate to specific serum proteins with certainty; quantitative studies were not undertaken. A protein present in the serum of the conceptus may be of either maternal or endogenous origin or both, but Melartin and her colleagues (2) demonstrated that the transferrin present in the conceptus may be of an allotype different from that of the mother as early as 9 wk gestation indicating synthesis of the protein by the embryo; similarly,

Hirschfeld and Lunell (3) found a haptoglobin allotype other than that of the mother in fetal serum of 17 wk gestation, while Mårtensson and Fudenberg also demonstrated that umbilical cord serum may contain γG molecules of an allotype different from that in maternal serum (4). Adapting the technique of Hochwald, Thorbecke, and Asofsky (5), van Furth, Schuit, and Hijmans (6) cultured fetal tissues in labeled amino acids and noted, by radioimmunoelectrophoresis of the culture fluids, that synthesis of prealbumin, albumin, α_2-macroglobulin, and transferrin occurs as early as 13 wk gestation, whereas γG and γM synthesis do not occur until 19 wk gestation. Using the same method, α-fetoprotein synthesis was demonstrated in the human embryo of 6 wk gestation (7).

A sustained or increasing serum concentration of a protein in the neonate during the immediate postnatal period suggests that the protein has been synthesized by the fetus: Hitzig has used this method to show that the full-term fetus can synthesize a number of plasma proteins (8). Conversely, a fall in the serum concentration of a protein after birth at a rate equal to the half-life of the protein suggests either a cessation of synthesis (9) or a lack of synthesis (10) of the protein by the fetus.

In the study reported here, the development of γG, γA, γM, β_{1C}/β_{1A}, C'1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, α_1-antitrypsin, orosomucoid, β-lipoprotein, α_2-macroglobulin, and prealbumin was investigated by culturing tissues from human embryos and fetuses of 29 days to 18 wk gestation, and by quantitation in serum from conceptuses of 5.5–41 wk gestation, in amniotic fluid between 6.5 and 38 wk gestation, as well as in serum from neonates during the 1st 3 wk of life.

METHODS

Tissues and tissue cultures. The human embryos and fetuses studied by means of tissue culture are listed in Table I; the synthesis of protein hormones of the anterior pituitary and placenta and the synthesis of thyroglobulin in these same conceptuses have been reported elsewhere (11). All of the conceptuses appeared to be grossly normal, the pregnancies having been therapeutically aborted for psychological reasons. Gestational age was estimated from Streeter's data (12) for embryos with a crown-rump length of 30 mm or less, and Patten's data (13) was used for crown-rump lengths of 35 mm or more. Conceptuses of 8.5 wk gestation or less were delivered per vagina by cervical dilatation followed by careful forceps extraction; those of 9.5 wk gestation or more were delivered by hysterotokotomy. The methods used for culture and radioimmunoelectrophoresis were essentially those described by Hochwald, Thorbecke, and Asofsky (5). Selected tissues were minced with scissors and up to 250 mg of tissue were incubated in roller tubes for 2–4 day at 37°C with 2 ml of Eagle's basal medium containing Hank's solution[1] and 2–4 μc of amino acids uniformly labeled with ^{14}C; tissues from conceptuses with identification codes beginning with C were incubated with L-leucine-^{14}C, and those with codes beginning with H were incubated with a mixture of amino acids obtained from ^{14}C-labeled algal hydrolysates. The cultures were then frozen and thawed twice, dialyzed against water for 3 days, lyophilized, and reconstituted to approximately 0.1–0.2 ml with 0.1 M borate buffer, pH 8.6. Immunoelectrophoresis (14) of the culture fluids, to which either normal adult serum, hypergammaglobulinemic serum, or serum from fetus No. 6 (Table II) were added as carriers, was performed in agar, allowing the slides to develop with antisera for 3–7 days, after which the slides were washed in 0.1 M NaCl for 2–3 days, dried, and then inverted on RS Pan[2] film for 4–12 wk for radioautography using lead bricks on top of the slides as weights. All determinations were performed in duplicate and occasionally in triplicate, and for some proteins such as γG, γA, and γM, at least two different antisera were used for each protein studied. In all but two instances individual organs or tissues were cultured separately from other tissues: the whole 6.4-mm embryo, which weighed less than 0.5 g, was minced and cultured to avoid possible loss of specific organs during dissection, and in C11-68 the abdominal viscera were minced together and cultured. Umbilical cord blood from C7-68 and C1-68 were also incubated with medium containing labeled amino acids and studied in the same manner as were the minced tissues. As controls, normal adult serum was added to Eagle's medium with and without the labeled amino acids, and studied by radioimmunoelectrophoresis for assurance that the precipitation line obtained with unlabeled protein did not give a false positive result on radioautography and that the unlabeled protein did not bind the labeled amino acids too firmly to be removed by the dialysis procedure. Non-

TABLE I

Conceptuses Studied by Tissue Culture in ^{14}C-Labeled Amino Acids

Conceptus	Crown-rump length	Estimated gestation
	mm	*wk*
C8-68	6.4	4.2*
C2-68	10	4.5‡
C4-68	20	5.5
H1-67	30	7.5
C3-68	35	8.5
C11-68	35	8.5
H6-67	45	9.5
H10-67	59	10.5
H2-67	70	11.5
C7-68	70	11.5
C10-68	79	12
H8-67	111	14
H9-67	145	17
C1-68	155	17.5
H3-67	160	18

* 29 days gestation.

‡ 32 days gestation.

[1] Grand Island Biological Co., Grand Island, N. Y.

[2] Eastman Kodak Co., Rochester, N. Y.

specific precipitation reactions of the concentrated tissue culture fluids with normal rabbit serum on immunoelectrophoresis as described by Tomasi (15) were also investigated, and were not seen in this study.

Sera, amniotic fluids, and quantitation of specific proteins. Umbilical cord blood was obtained from five of the conceptuses listed in Table I as well as 13 additional conceptuses delivered by therapeutic abortion between 5.5 and 26 wk gestation (Table II). Blood was also obtained, either from the umbilical cord or by heel puncture at birth, from 21 infants delivered after the spontaneous onset of labor or by hysterotokotomy between 27.5 and 41 wk gestation, and from seven mothers by venipuncture at the time of delivery (Table II). Amniotic fluid was obtained from 10 conceptuses (Table III); the fluids were concentrated 20–40 times by dialysis against water, lyophilization, and reconstitution to the reduced volume with 0.15 M NaCl.

Quantitation of specific proteins in these sera and in the amniotic fluids was performed by radial immunodiffusion (16) employing antisera specific only for the protein being estimated. The amounts of sera and amniotic fluids available were quite limited, particularly from the smaller embryos, so that not all proteins could be determined in each serum or fluid. In the absence of sufficient amounts of purified proteins, the serum and amniotic fluid concentrations of most of the specific proteins determined (Tables II and III) were related instead to the concentration of the given protein present in a pool of normal adult serum which was used as the reference standard. The purified preparations of γG, ceruloplasmin, and transferrin used as the primary reference standards for the estimation of these proteins were obtained by low temperature ethanol fractionation of pooled normal adult plasma (17–19). The purified γA preparation used as the reference standard was isolated from pooled human serum by Sephadex[3] G200 filtration followed by DEAE-cellulose column chromatography using a gradient from 0.001 M phosphate buffer, pH 7.4, to 0.05 M phosphate buffer, pH 7.4, as described elsewhere (20). Immunoelectrophoresis and double-gel diffusion with specific antisera revealed the presence of γA in this preparation but no detectable γG or γM; when radioiodinated with ^{131}I (21), 82% of the protein-bound radioactivity of the labeled γA preparation was precipitable with antisera specific for γA, and the γA content of the preparation was taken to be 82% of the total protein in the preparation. The γM reference standard was a preparation isolated by filtration of pooled human serum through a column of Sephadex G200 followed by Geon[4]-block electrophoresis (22); when radioiodinated with ^{131}I (21) approximately 80% of the protein-bound radioactivity of this preparation was precipitable with specific antisera against γM, and the γM content of the preparation was taken to be 80% of the total protein present in that preparation. The β_{1C}/β_{1A} reference standards used were commercially[5] available human sera of given β_{1A} concentrations expressed in terms of β_{1C}; the primary β_{1A} reference standard used to establish the β_{1A} concentrations in these sera was prepared[6] by a modification of the method of Steinbuch, Quentin, and Pejaudier (23).

The rate of diffusion of a specific protein in agar, and hence the diameter of the precipitin ring obtained with a given concentration of the protein by radial immunodiffusion, is in part dependent upon the molecular size of the protein. Since γA in secretions is usually of a higher molecular weight than that in plasma, an estimate of the relative molecular size of amniotic fluid γA was made employing the principle of Martin and Ames (24) using ultracentrifugation in a sucrose gradient prepared in a 1×5 cm tube as described by Kunkel, Rockey, and Tomasi (25), the sucrose gradient being 5 g/100 ml at the top and 25 g/100 ml at the bottom. Concentrated amniotic fluid, the purified serum γA which had a sedimentation coefficient of approximately 7S and a purified preparation of 11S γA isolated from human saliva by filtration through Sephadex G200 and Sephadex G100 followed by gradient elution through DEAE-cellulose columns were centrifuged separately in the sucrose gradient tubes at 105,000 *g* in a swinging bucket of the Spinco Model L ultracentrifuge for 18 hr. The bottom of each tube was then pierced with a No. 25 needle and the contents collected in 0.3-ml aliquots and assayed for γA by radial immunodiffusion. Under these conditions, the 11S salivary γA was found at the bottom of the tube, whereas the amniotic fluid γA was found in the middle portion of the tube at a position similar to that observed for serum γA. Thus, amniotic fluid γA and serum γA appeared to be similar in molecular weight. In this same regard, since β_{1C} is converted to β_{1A} on storage, and β_{1A} is a smaller molecule than β_{1C}, it should be noted that under the conditions of storage in this study all of the β_{1C}/β_{1A} detectable by immunoelectrophoresis of amniotic fluids, embryonic, fetal, and maternal sera migrated as β_{1A} just as in the pooled sera used as the β_{1C}/β_{1A} reference standards.

Antisera. Antisera specific for either γG, γA, or γM were prepared in rabbits using complete Freund's adjuvant. The antisera against γG were adsorbed with 0.1–0.2 volumes of serum from a child with agammaglobulinemia to neutralize the small amounts of antibodies present in the antisera against serum proteins other than γG. Some of the antisera against γA contained precipitins against γG as well as γA; these antisera were made specific for γA alone by adsorption with serum from a patient with ataxia telangiectasia who lacked detectable serum γA. The rabbit antisera against γM contained precipitins for serum α_2-macroglobulin and γG, but were readily made specific for γM by adsorption with serum from a patient with congenital spherocytosis who lacked γM. The rabbit antiserum against ceruloplasmin was adsorbed with serum from a patient with Wilson's disease who was deficient in ceruloplasmin; it was the same antiserum used in an earlier study (26). The preparation and immunochemical characteristics of the rabbit antiserum against transferrin have been described elsewhere (27); this antiserum was used unadsorbed or adsorbed with albumin and γG protein fractions to render it specific for transferrin. Also described elsewhere is the preparation of the rabbit antisera against fibrinogen (28), β-lipoprotein (27), and fetal serum proteins (7, 9). Antiserum against α_2-macroglobulins was prepared in rabbits using Freund's complete adjuvant and a purified preparation of α_2-macroglobulin isolated by Sephadex G200 filtration and Geon-block electrophoresis (22). Antisera against adult serum proteins were also prepared in rabbits using Freund's complete adjuvant. Rabbit antisera specific for either β_{1C}/β_{1A}, C'1-esterase inhibitor, hemopexin, haptoglobin, plasminogen, α_1-antitrypsin, and orosomucoid were obtained commercially;[5, 6] each of these antisera gave but a single line on immunoelectrophoresis against normal adult serum.

Peptide studies on β-lipoprotein and α_2-macroglobulin formed in liver culture. Adult serum, used as a source of

[3] Pharmacia, Uppsala, Sweden.

[4] The B. F. Goodrich Company, Cleveland, Ohio.

[5] Hyland Laboratories, Costa Mesa, Calif.

[6] Behringwerke, Marburg-Lahn, Germany.

TABLE II

Concentrations of Specific Proteins

Conceptus	Estimated gestation	γG	γA	γM	β_{1C}/β_{1A}	C'1-esterase inhibitor	Ceruloplasmin
	wk	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	%‡	*mg/100 ml*
C9-68	5.5	66	*	*	1.9	*	*
5	6.5	70	1.0	0	*	20	0.4
H11-67	7.5	52	*	0	1.9	*	*
4	9.5	60	10	0	5.5	25	3.3
H10-67	10.5	73	0§	0	4.7	25	1.0
C7-68	11.5	59	0.2	0	5.9	*	2.4
C5-64	13	56	0.2	0	7.9	35	0.8
H8-67	14	52	0	0	2.4	37	0
H5-67	15	77	2.2	0	8.0	20	2.8
H9B-67	15.5	77	0.2	0	8.6	38	3.5
H4-67	17	102	1.5	1.2	9.2	20	3.5
C1-68	17.5	178	0	0	15	37	1.5
6	18	105	0	0	8.6	33	0
H3-67	18	136	0	0	8.0	40	3.1
1	20	180	*	*	17	*	*
2	21	110	0	0	8.6	33	0
7	22	136	0	0	10	33	0.8
J3-65	26	1450	0	0	47	40	0
SC	27.5	1200	*	*	108	105	17.5
P12	30	920	0	0	98	183	15.5
CO‖	35	700	0.2	1.2	108	100	8.6
P11	36	1100	0	0	152	*	8.0
P18	36	1400	*	*	58	89	12.0
P1	37	*	0	1.2	167	*	5.1
GR	37	900	0	1.5	58	*	9.4
N1‖	38	1340	0.2	1.2	146	125	6.7
MC‖	38	2200	0	0	96	125	5.7
HAG‖	39	2200	0	1.2	108	140	8.6
HAT‖	39	*	0	9.3	78	100	6.7
BL	39	1180	0.2	0	87	100	13.5
JE	39	2300	0	3.8	67	*	3.5
BA-1‖	40	1180	1.7	1.2	119	105	14.5
BA-2‖	40	*	0	1.2	108	105	12.0
HO‖	41	*	0	0	118	*	4.6
SW	41	*	0	0	146	100	15.5
Maternal¶		*	213±28	77 ±10	161 ±9	147 ±33	50.0 ±5.4
Limit of sensitivity		10	0.2	1.2	0.5	2	0.2

* Not done.

‡ As per cent of level in a normal adult serum pool; absolute concentration not determined.

§ 0 = below limit of sensitivity of method which is given at bottom of each column.

‖ Sera from the mothers of these infants were obtained at same time; the maternal values are given in the next to last line of table.

¶ Mean ±SD.

carrier β-lipoprotein and α_2-macroglobulin, was added to approximately 25 μl of the supernatant liver culture fluid of C3-68, one of the embryos of 8.5 wk gestation. The culture fluid was diluted to 1 ml with 0.15 M NaCl; β-lipoprotein and α_2-macroglobulin were then precipitated separately in the zone of antibody excess with specific antisera. The specific precipitates were washed three times with 0.1 M NaCl resuspended in distilled H_2O, heated to 100°C for 1 hr, and then hydrolyzed at 37°C with α-chymotrypsin in a pH stat (29) using an enzyme to protein ratio of 1:200. The resulting peptides were separated by high voltage electrophoresis on Whatman No. 1 filter paper cooled by Varsol[7] as described elsewhere (30). The papers were dried, placed on sheets of RS Pan film for 8 wk for detection of radioactive peptides. The radioautograph patterns found were

[7] Humble Oil & Refining Co., Houston, Tex.

Transferrin	Hemopexin	Haptoglobin	Plasminogen	α_1-Antitrypsin	β-Lipoprotein	α_2-Macroglobulin
mg/100 ml	%‡	%‡	%‡	%‡	%‡	%‡
*	*	*	*	*	*	*
*	*	*	*	6.5	*	*
28	*	0	*	*	*	*
*	*	1.7	*	56	49	17
43	2.9	0	23	80	95	14
41	5.0	2.5	43	86	49	14
43	3.2	1.0	42	70	37	17
34	3.2	0	18	56	57	20
47	4.9	2.7	10	56	36	14
60	2.9	0	43	92	42	34
47	4.0	0	10	56	36	26
65	4.4	0	8	80	33	40
60	7.2	0	30	80	33	29
47	2.9	0	23	80	42	29
99	*	*	*	73	*	*
45	2.9	0	18	56	9	34
49	1.0	0	14	56	25	42
*	5.3	0	23	92	42	83
225	*	1.0	*	195	42	210
205	10	*	77	158	9	180
135	16	0	54	110	21	129
150	16	*	60	115	7	125
160	*	*	*	*	9	215
175	29	2.1	110	135	12	139
225	35	*	*	110	9	120
98	33	1.8	77	135	5	159
210	15	1.8	30	135	14	200
98	42	1.8	60	135	42	129
150	31	*	18	163	36	159
190	19	*	*	135	9	159
225	29	*	*	115	31	103
265	38	*	*	123	31	159
225	27	*	*	163	31	210
175	33	*	*	146	21	159
265	29	*	*	175	20	190
305 ±48	163 ±20	94 ±3	205 ±43	205 ±14	87 ±2	103 ±7
1.0	1.0	1.0	2.5	0.5	2.5	5.0

compared to the ninhydrin peptide patterns for similar enzymatic hydrolysates of the purified proteins.

RESULTS

γG. The earliest stage in which radioactive γG was consistently found in tissue culture was at 12 wk gestation in cultures of liver and gastrointestinal tract, the latter culture including mesenteric lymph nodes as well; the spleen was not cultured in this fetus. The largest amounts of radioactive γG were found in cultures of the spleen from the fetuses of 17–18 wk gestation (Fig. 1); small amounts of radiolabeled γG were detected in the thymus cultures of C1-68 and H3-67, or 17.5 and 18.0 wk gestation, respectively, and in the liver culture of

TABLE III

Concentrations of Specific

Conceptus	Estimated gestation	γG	γA	γM	β_{1C}/β_{1A}	C'1-esterase inhibitor
	wk	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	%‡
5	6.5	0§	*	0	0.13	*
C3-65	9.5	1.7	0	0	*	2.9
4	9.5	4.1	0.14	0	0.25	*
H6-67	9.5	44.0	1.90	0	1.52	4.1
H10-67	10.5	16.5	0.39	0	0.31	1.3
C7-68	11.5	11.0	0.14	0	0.28	0.5
C5-64	13	17.5	0.34	0	0.31	1.3
C1-68	17.5	36.5	1.25	0	1.25	1.3
WE	38	9.0	*	0	1.70	0.9
CHP66	38	8.9	0.42	0	0.33	0.9

* Not done.

‡ As per cent of level in normal adult serum pool.

§ 0 = below limit of sensitivity of method with amniotic fluid concentrated 20–40 times; limits of sensitivity given at bottom of Table II.

C1-68. Cultures of cells from approximately 0.2 ml of umbilical cord blood from C1-68 contained an amount of radioactive γG almost equivalent to that seen in the spleen culture of H9-67. Cultures of cells from 0.4 ml of maternal venous blood contained readily detectable amounts of radioactive γG.

Between 5.5 and 22 wk gestation, the serum γG level increased only slowly (Fig. 2), ranging from 52 to 180 mg/100 ml (Table II). By 26 wk gestation, however, the serum γG level had increased dramatically (Fig. 2), ranging from 700 to 2300 mg/100 ml between 26 and 40 wk gestation. The concentration of γG in amniotic fluid from conceptuses of 9.5–38 wk gestation was from 1.7 to 44.0 mg/100 ml (Table III).

γM. Radiolabeled γM was first readily and consistently detected at 10.5 wk gestation. Radiolabeled γM was also evident in the spleen cultures of C7-68 and H9-67, or at 11.5 and 17 wk gestation, respectively, but the largest amounts were found at 17.5 and 18 wk gestation (Fig. 1); radioactive γM was also found in the thymus culture of C1-68 at 17.5 wk gestation, and in the blood cell incubation fluids of C7-68, C1-68, and the mother of C4-68. None of the other cultures of individual tissues yielded detectable amounts of radioactive γM.

Most of the embryonic and fetal sera did not contain detectable amounts of γM (Fig. 2), but it should be emphasized that the lower limit of sensitivity of the method for γM in this study was 1.2 mg/100 ml (Table II). A fetus of 17 wk gestation did have 1.2 mg/100 ml of γM, but all the remaining sera were negative until 35 wk gestation. Although the amniotic fluids were concentrated 20–40 times, γM was not detected in any of these fluids (Table III).

γA. Radioactive γA was not found in any of the cultures; the cultures examined were the same as those studied for the presence of radiactive γG and γM.

The serum concentrations of γA in the younger conceptuses were higher than those delivered during the last half of the gestational period (Fig. 2), and immunoelectrophoresis confirmed the fact that the protein being measured was indeed γA. Only one of the eight amniotic fluids studied did not contain detectable γA (Table III); the concentration of γA in the other amniotic fluids ranged from 0.14 to 1.90 mg/100 ml. Interestingly, two of the amniotic fluids, those of H10-67 and C1-68, contained γA when the serum of the same conceptuses (Table II) did not contain detectable γA, the lower limit of detection for γA in these sera being 0.2 mg/100 ml.

β_{1C}/β_{1A}. Radioactive β_{1C}/β_{1A} was detected in almost every fluid, beginning with those of the embryo of 4.2 wk gestation. Cultures of lung yielded the most radioactive β_{1C}/β_{1A}, but cultures of skin, subcutaneous tissue, and muscle also yielded β_{1C}/β_{1A} of high radioactive content as did cultures of the liver and the placenta. Cultures of blood from C7-68 and C1-68 contained trace amounts of radioactive β_{1C}/β_{1A}.

The serum concentration of β_{1C}/β_{1A} rose steadily (Fig. 2) from a low of 1.9 mg/100 ml at 5.5 wk gestation to a range of 52–167 mg/100 ml between 28 and 41 wk gestation (Table II and Fig. 2). The maternal serum levels of β_{1C}/β_{1A} ranged from 131 to 200 mg/100 ml, and in each instance the serum concentration in the mother was greater than that in her infant, the difference being from 13 to 104 mg/100 ml. Postpartum (Fig. 2), there was a tendency for the neonatal serum concentration of β_{1C}/β_{1A} to decrease only slightly over the 1st 2 wk after birth; the concentration of β_{1C}/β_{1A} in the infants of 34–36 wk gestation during the 1st 3 wk of life

Ceruloplasmin	Transferrin	Hemopexin	Haptoglobin	Plasminogen	α_1-Antitrypsin	β-Lipoprotein	α_2-Macroglobulin
mg/100 ml	*mg/100 ml*	%‡	%‡	%‡	%‡	%‡	%‡
*	*	*	*	*	0.10	0	0
0.04	0.54	0.42	0.39	0	0.90	0	0
0.80	17	2.2	0	4.0	8.1	0	0
0	0.83	0	0	0	0.93	0	0
0.37	7.0	0.53	0	0.5	8.5	0	0
0.37	17	1.9	0.40	0.5	6.8	0	0
0.19	7.0	0.62	0	0.5	6.2	0	0
0.19	40	0.79	0.31	2.4	11.7	0	0
0.28	17	*	*	*	7.7	0	0
0.30	2.9	0.37	0	2.7	5.5	0	0

ranged between 52 and 182 mg/100 ml. All of the amniotic fluids studied contained β_{1C}/β_{1A}, the concentrations ranging from 0.13 to 1.70 mg/100 ml (Table III).

C'1 esterase inhibitor. As with β_{1C}/β_{1A}, almost all of the cultures yielded radioactive C'1 esterase inhibitor, including those of the placenta. Unlike β_{1C}/β_{1A}, however, radioactive C'1 esterase inhibitor was not found in any of the blood cultures. The serum concentration of C'1 esterase inhibitor at 6.5 wk gestation was 20% of that in the normal adult serum pool used as the reference standard; the concentration increased to reach almost adult levels by 28 wk gestation. There was no correlation evident between maternal and infant levels of the protein in the paired maternal-infant sera: some infants had concentrations at birth which were higher than those seen in the mother, and others had concentrations which were lower. All amniotic fluids studied contained C'1 esterase inhibitor, the concentration of the protein being between 0.5 and 4.1% of the adult serum pool used as the reference standard (Table III).

Ceruloplasmin. Radioactive ceruloplasmin was found in all of the liver cultures studied with but few exceptions: ceruloplasmin could not be detected in the culture of the embryo of 4.2 wk gestation nor in the liver culture of an 11.5 wk embryo, H2-67, although, as will be noted, synthesis of other radioactive proteins in these same cultures was evident. Synthesis of ceruloplasmin was not detected in cultures of lungs, thymus, spleen, gastrointestinal tract, kidneys, pancreas, skeletal muscle, or placenta, nor did synthesis of ceruloplasmin take place in the fetal or maternal blood cultures.

The serum concentration of ceruloplasmin was below the limit of sensitivity of the method, 0.2 mg/100 ml, in four fetuses between 14 and 26 wk gestation (Table II); in the others, serum ceruloplasmin was only 0.4 mg/100 ml at 6.5 wk gestation and reached a range of 4.6–15.5 mg/100 ml during the period of 27.5–41 wk gestation (Table II and Fig. 3). There appeared to be some decrease in serum ceruloplasmin postpartum (Fig. 3). The ceruloplasmin concentration in seven mothers (Table II) ranged from 33 to 73 mg/100 ml and aver-

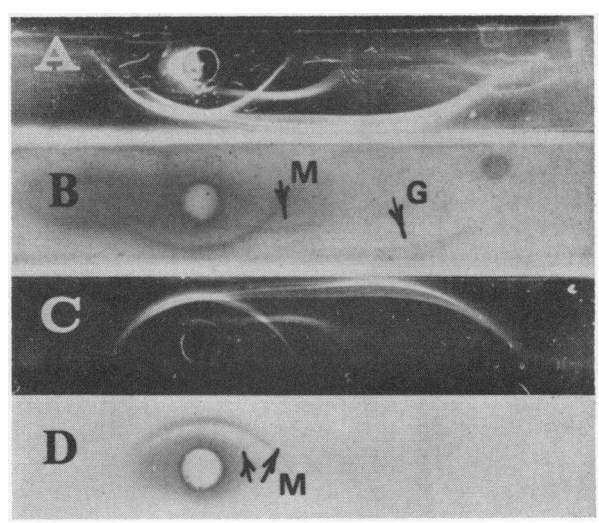

FIGURE 1 A. Immunoelectrophoresis of spleen culture from H3-67, 18 wk gestation, using hypergammaglobulinemic serum as carrier and developed with unadsorbed anti-γM. B. Radioautograph of A; arrow *M* indicates γM line and arrow *G* marks the γG line; semicircular band around antigen well is α_2-macroglobulin. C. Immunoelectrophoresis of C7-68 spleen culture, 11.5 wk gestation, using hypergammaglobulinemic serum as carrier and developed with unadsorbed anti-γM. D. Radioautograph of *C*; γM line outlined by arrows. Anode was to the left.

aged 50 ±5.4 mg/100 ml. Amniotic fluid ceruloplasmin levels were much lower than those in serum, at least for those conceptuses where both amniotic fluid and serum were obtained (Table III).

Transferrin. Radioactive transferrin was found in each liver culture or culture containing liver. Traces of radioactive transferrin were found in the lung cultures of three embryos between 8.5 and 12 wk gestation, but not in lung cultures of six other conceptuses from 7.5 to 18 wk gestation. It was not detected in any of the cultures of thymus, spleen, gastrointestinal tract, kidneys, pancreas, placenta, or blood. Radioactive transferrin was found in large quantities, however, in cultures of yolk sacs obtained from four embryos between 5.5 and 11.5 wk gestation.

Serum transferrin between 6.5 and 22 wk gestation ranged from 28 mg/100 ml to 99 mg/100 ml (Table II and Fig. 3); between 27.5 and 41 wk gestation, the concentration range was 98–265 mg/100 ml. There were no dramatic changes in serum transferrin in the postnatal period (Fig. 3). Maternal serum concentrations of transferrin were between 195 and 550 mg/100 ml, with an

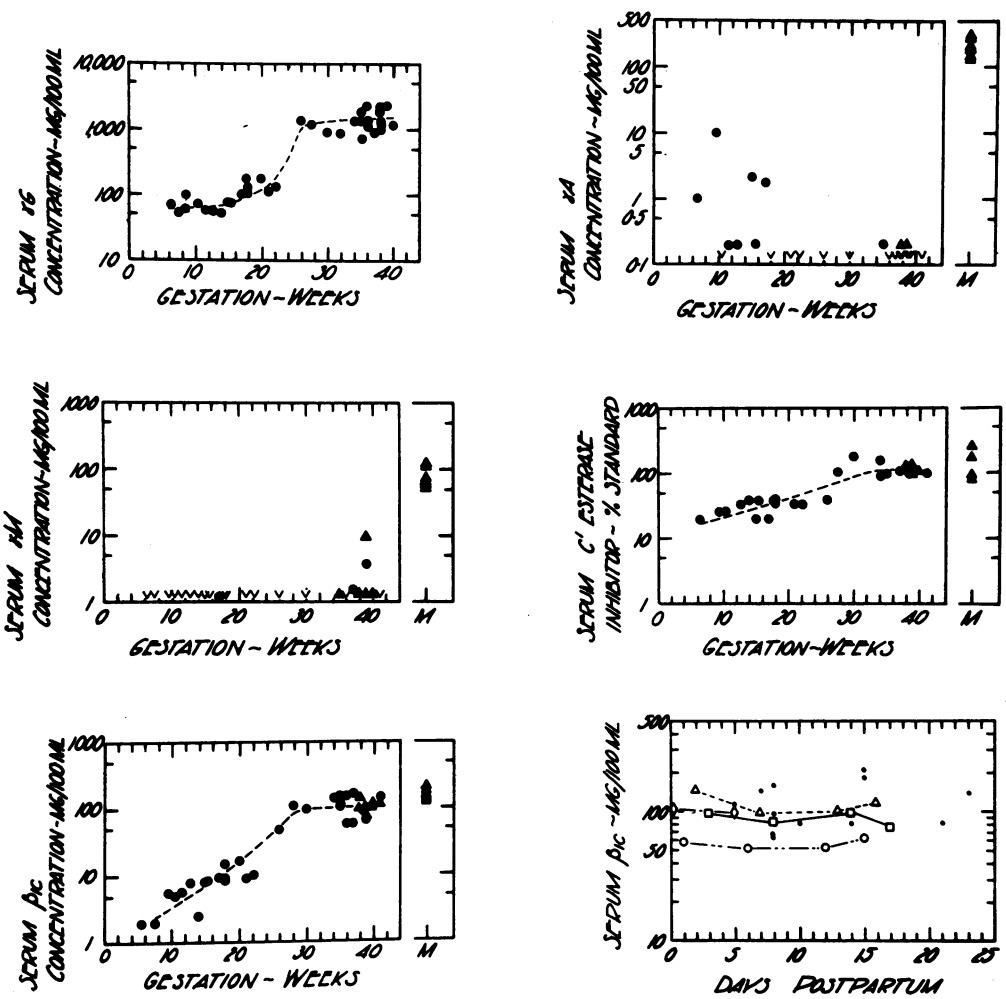

FIGURE 2 Embryonic and fetal serum concentrations of γG, γA, γM, C'1 esterase inhibitor, and β_{1C}/β_{1A}, and neonatal serum concentrations of β_{1C}/β_{1A} in infants of 34–36 wk gestation during the 1st 23 days postpartum; the "v" marks along the abscissae of these graphs relating serum γA and serum γM to gestational age indicate that the serum concentration of γA or γM, respectively, in the given conceptus was below the limit of sensitivity of the method for the specific protein. Solid triangles (▲) over abscissa marked *M* are the serum concentrations in the mothers of those fetuses indicated by solid triangles immediately to the left. Postpartum serial serum concentrations of β_{1C}/β_{1A} from the same neonate in graph at lower right are indicated by connected open symbols. It should be noted that the ordinates are logarithmic in these and all subsequent graphs.

average of 305 ±48 mg/100 ml. In each instance, the maternal transferrin concentration was higher than that in the infant (Fig. 3), the difference between them ranging from 90 to 400 mg/100 ml, the average of the differences being 159 mg/100 ml. The amniotic fluid transferrin levels are given in Table III.

Hemopexin. Radioactivity associated with the hemopexin precipitation line was present in all of the cultures

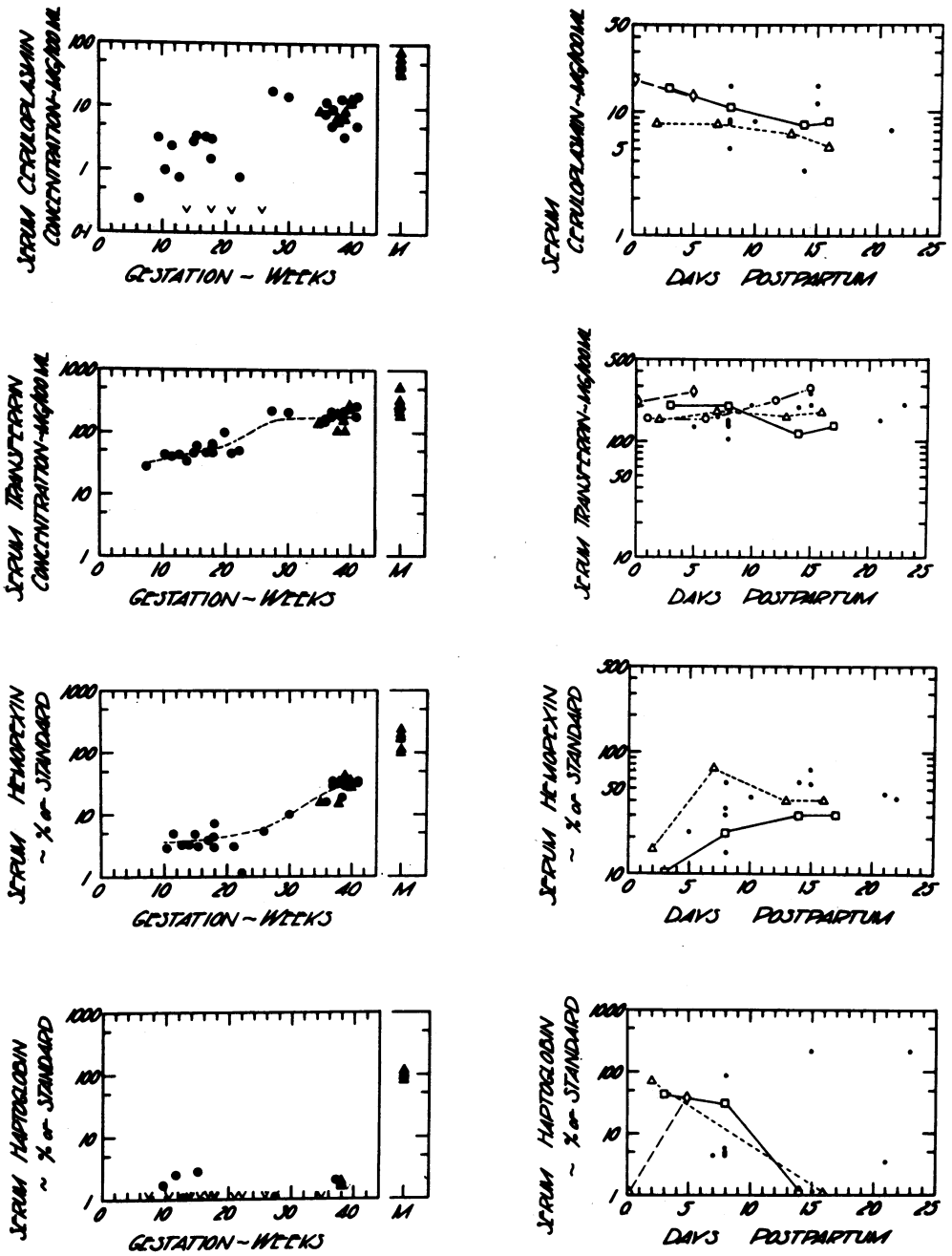

FIGURE 3 Embryonic, fetal, and neonatal serum concentrations of ceruloplasmin, transferrin, hemopexin, and haptoglobin. Embryonic and fetal data are on graphs to the left; a "v" indicates that the serum concentration of the protein in that conceptus was below the limit of sensitivity of the method. Neonatal data are on graphs to the right as dots or connected open symbols; serial measurements from a given infant are indicated by the connected symbols.

containing liver; it was not found in cultures of the spleen, gastrointestinal tract, or kidneys. Cultures of lungs contained small amounts of radioactive hemopexin. Radioactive hemopexin was not found in the fetal blood cultures, although the maternal blood cultures did contain trace amounts.

Hemopexin was present in embryonic serum as early as 10.5 wk gestation; serum from earlier embryos was not studied (Table II). The range for serum hemopexin between 10.5 and 26 wk gestation was 1.0–7.2% of the concentration in the adult serum pool (Table II and Fig. 3). Between 30 and 41 wk gestation, serum hemopexin ranged from 10 to 42% of that in the adult pool. Postnatally, there was an apparent increase in serum hemopexin (Fig. 3). Maternal serum hemopexin at delivery was much higher than that in the infant (Table II and Fig. 3). The concentrations of hemopexin in amniotic fluid (Table III) were lower than those in the sera.

Haptoglobin. The haptoglobin precipitation line obtained by immunoelectrophoresis of normal adult serum *without* added radioactive amino acids often gave a dark line on the radioautography film which was indistinguishable from that found on radioimmunoelectrophoresis of the liver culture fluids for radioactive haptoglobin. Therefore, the radioimmunoelectrophoresis data for haptoglobin in tissue cultures could not be interpreted. The ability of haptoglobin to affect the silver grains of the photographic film was not investigated further, except to ascertain that this property was not shared by hemoglobin which is bound by haptoglobin.

Small amounts of haptoglobin were found in 9 of the 21 sera of 7.5–39 wk gestation (Table II and Fig. 3). The postnatal serum haptoglobin concentrations ranged from a low of 1% of that in the adult serum pool to as much as 230% of the adult pool (Fig. 3); serum haptoglobin rose rapidly in one infant in the immediate postnatal period, but fell in others, the shifts in concentration being quite dramatic in extent. Maternal serum haptoglobin levels were from 80 to 100% of that in the normal adult pool (Table II and Fig. 3). Three of the eight amniotic fluids examined contained low concentrations of haptoglobin (Table III).

Fibrinogen. Synthesis of radioactive fibrinogen was first detected at 5.5 wk gestation. It was not found at 4.2 or 4.5 wk gestation nor in H1-67 of 7.5 wk gestation, but all other liver cultures studied contained radioactive fibrinogen.

Since all bloods collected for this study were permitted to clot, plasma fibrinogen could not be estimated.

Plasminogen. Insufficient amounts of tissue culture concentrates precluded a study of the tissue sites of plasminogen synthesis. Embryonic, fetal, and maternal serum concentrations of plasminogen in terms of the level present in the normal adult serum pool are given in Table II and Fig. 4. Serum plasminogen tended to be lower earlier in gestation, but between fetuses of similar gestational age the highest plasminogen concentration was as much as four to five times that of the lowest. Maternal plasminogen in each instance was at least twice that of the infant. Six of the eight amniotic fluids examined contained detectable but low concentrations of plasminogen (Table III).

α_1-Antitrypsin. Radioactive α_1-antitrypsin was found in all cultures containing liver. Trace amounts of radioactive α_1-antitrypsin were observed in the fetal and maternal blood cultures, but the amounts present in the liver cultures far exceeded that in the blood cultures. Other tissue cultures were not studied; the radioimmunoelectrophoresis controls consisting of the carrier serum alone with and without added labeled amino acids were negative for α_1-antitrypsin.

Serum α_1-antitrypsin was quite low in the embryo of 6.5 wk gestation, but by 9.5 wk gestation the concentration was more than half of that in the normal adult pool (Table II and Fig. 4). In all but one instance, the serum concentration of α_1-antitrypsin was lower in the infant than in the mother. Serum α_1-antitrypsin levels during the postnatal period tended to be lower than those seen at birth (Fig. 4). Amniotic fluid contained low concentrations of α_1-antitrypsin (Table III).

Orosomucoid. Radioactive orosomucoid, or α_1-acid glycoprotein, was detected as early as 4.5 wk gestation; none was detected in the embryo of 4.2 wk gestation. All other liver cultures contained radioactive orosomucoid; blood cultures and radioimmunoelectrophoretic controls were negative. Other tissues were not studied.

β-Lipoprotein. Radioactive β-lipoprotein was found in relatively large amounts in the fetal and maternal blood cultures. All other tissue cultures, including those of the 6.4 mm embryo, contained some radioactive β-lipoprotein but it could not be determined if the radioactive protein was synthesized by the tissue or by the blood cells in that tissue, even though the liver cultures contained the most radioactive β-lipoprotein. Enzymatic hydrolysis of the β-lipoprotein specific precipitate from the liver culture of C3-68 revealed some peptides which were radioactive, but the radioautography patterns were too faint other than to note that the radioactive peptides were primarily in two bands which corresponded in electrophoretic mobility to two major ninhydrin bands found with the purified β-lipoprotein hydrolysate. The hydrolysate of unlabeled purified β-lipoprotein and an α-chymotrypsin hydrolysate of unlabeled rabbit γG, which was the other protein in the specific precipitate, had no detectable effect on the radioautography film.

Interestingly, the serum concentrations of β-lipoprotein tended to be higher earlier in gestation than those seen

later in gestation (Table II and Fig. 4). Neonatal serum β-lipoprotein levels covered a wide range of values; the neonatal sera, however, were collected without reference to meals or time of day. Amniotic fluid did not contain detectable amounts of β-lipoprotein (Table III).

α_2-Macroglobulin. As with β-lipoprotein, relatively large amounts of radioactive α_2-macroglobulin appeared in the blood cultures as well as in all cultures containing liver, including the culture of the 6.4 mm embryo. The α_2-macroglobulin specific precipitate obtained with the liver culture of C3-68 yielded some peptides on hydrolysis which proved to be radioactive on radioautography. Although the radioautographs were very faint, it could be discerned that the radioactivity was present in at least two major bands which corresponded to ninhydrin bands of similar electrophoretic mobility found for the purified α_2-macroglobulin hydrolysate; the unlabeled purified protein hydrolysate had no detectable effect on the radioautography film.

Serum α_2-macroglobulin levels in the conceptus increased as gestation increased (Table II and Fig. 4), reaching levels which were greater than those seen in the mother at term and which were either sustained or decreased only slightly during the neonatal period (Fig. 4). Amniotic fluid did not contain detectable amounts of α_2-macroglobulin (Table III).

Prealbumin. Radioactive prealbumin was found in each of the cultures containing liver. It was not found in cultures of the lungs, gastrointestinal tract, spleen, kidneys, placenta, or blood. As with transferrin, however, it was present in relatively large amounts in cultures of the yolk sac of 5.5 wk gestation; less radioactive prealbumin was found in the yolk sac of 8.5 wk gestation, and none was detected in the yolk sac of 11.5 wk gestation which was already solid and atretic (31).

Serum and amniotic fluid concentrations of prealbumin were not determined.

DISCUSSION

The radioactivity of the plasma proteins found on radioimmunoelectrophoresis of the tissue culture fluids was not attributable to simple binding of labeled amino acids to carrier protein. Most of the cultures, other than those of liver, were negative for radioactive ceruloplasmin, transferrin, hemopexin, and prealbumin, although the incubating medium in these cultures contained the same labeled amino acids as those in the liver cultures. In addition, none of the proteins of this study retained radioactivity detectable by radioautography after incubation of the unlabeled protein with labeled amino acids followed by dialysis and immunoelectrophoresis. It is possible, of course, that the liver might synthesize a radioactive substance from the labeled amino acids that could be bound by each of the proteins, but since only a few of the proteins studied were intensely radioactive in the yolk sac cultures, one would have to postulate synthesis of still another radioactive substance by the yolk sac which is selectively bound by the latter proteins and not the others. Similarly, blood cultures yielded only a few radioactive plasma proteins as did the lungs, and these radioactive proteins were not necessarily the same as those found in the yolk sac. In the case of hemopexin which binds heme, it is doubtful that the radioactivity associated with hemopexin was radioactive heme, since even the blood and spleen cultures, both of which synthesized relatively large amounts of radioactive hemoglobin, were negative for radioactive hemopexin. Although it is possible that some of the radioactivity found in β-lipoprotein was attributable to binding of radioactive lipid by the protein moiety, at least some of the radioactivity would appear to be in the protein moiety in view of the finding that at least two peptide bands in the electrophoretic patterns for the radioactive β-lipoprotein hydrolysate were radioactive: the only labeled amino acid in the liver culture from which the protein was precipitated was leucine. Similary, the radioactive α_2-macroglobulin revealed labeled peptides on hydrolysis.

van Furth (6) has demonstrated that radiolabeled γG can be synthesized in cultures of fetal spleen beginning at 19 wk gestation; he was unable to detect synthesis of γG in the tissue cultures of any of four fetuses from 13 to 18 wk gestation. In the present study, however, relatively large amounts of radioactive γG were detected in tissue cultures from fetuses of 17–18 wk gestation and tissue cultures from an embryo of 12 wk gestation also yielded significant amounts of labeled γG. In addition, van Furth (6) did not detect labeled γG in any liver cultures from fetuses of 13–39.5 wk gestation, and did not find labeled γG in thymus cultures except in two fetuses of 22 and 33 wk gestation, respectively. In the present study, radioactive γG was detected in cultures of liver, thymus, and gastrointestinal tract of one or more conceptuses between 12 and 18 wk gestation. Apparently, synthesis of γG can begin much earlier in human development than has previously been observed, and in organs other than the spleen even during this early period. The marked immaturity in morphologic development of the conceptus of 12 wk gestation compared with that of the fetus of 19 wk gestation need hardly be emphasized, but it may be noted, for example, that lymphoid cells in the 12 wk embryo are present in the thymus and mesenteric nodes and are absent or only sparsely present in the spleen, appendix, tonsils, and peripheral blood (32), whereas lymphoid cells in the 19 wk fetus are readily found in all of these organs, plasma cells can sometimes be distinguished, and Hassall's bodies, a site where γG is found in children (27), may be evident in the thymus (32).

Since γG synthesis either was not detected or was relatively low in tissue cultures of conceptuses between 4.2 and 17 wk gestation, it appears likely that most of the γG present in embryonic and fetal serum between 5.5 and 17 wk gestation was maternal in origin. Indeed, Dancis and his colleagues (33) have demonstrated that γG is transferred from mother to fetus at 3 months gestation. Although increased fetal γG synthesis may account for some of the increase in the fetal serum γG level seen after 17 wk gestation, endogenous γG synthesis even in the full-term infant can account for only a small fraction of the serum γG present at delivery (8, 10, 34). The marked increase in fetal serum γG noted between 22 and 26 wk gestation, therefore, must be owing primarily to a rapid increase in the rate of maternofetal transfer of γG.

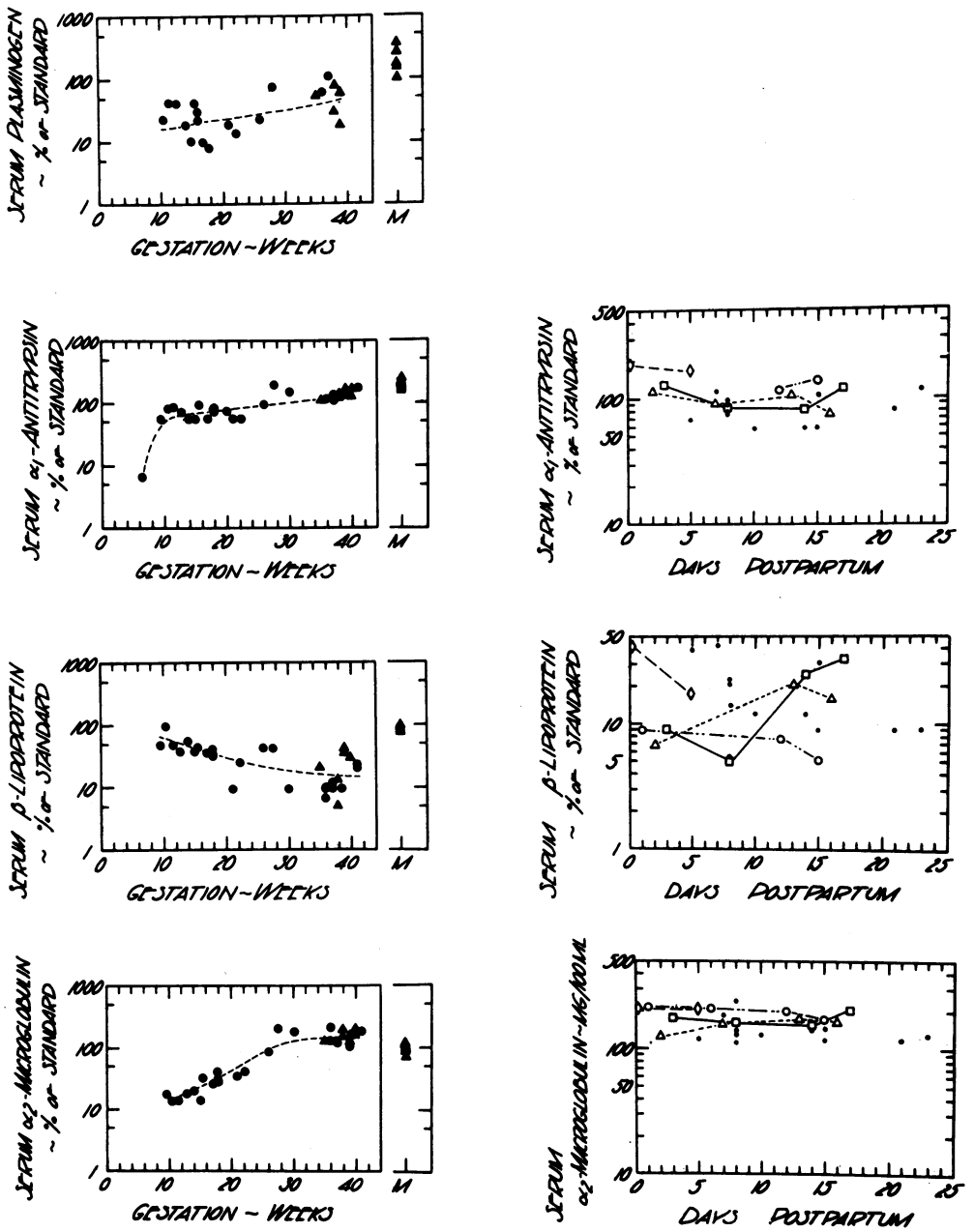

FIGURE 4 Serum concentrations of plasminogen, α_1-antitrypsin, β-lipoprotein, and α_2-macroglobulin. Embryonic and fetal data are on graphs to the left. Neonatal data are on graphs to the right; serial measurements from the same infant are indicated by connected open symbols.

Radiolabeled γM was definitely present in cultures beginning at 10.5 wk gestation, the greatest amount being observed at 17.5 and 18 wk gestation, the oldest fetuses studied. Although van Furth reported the synthesis of γM in spleen cultures of fetuses as young as 19 wk gestation, he did not detect radiolabeled γM in fetuses between 13 and 18 wk gestation (6). Some of the differences between the data obtained in the present study and those of van Furth may be attributable to the fact that the cultures in the present study were incubated longer, larger amounts of the concentrated culture fluids were used for each immunoelectrophoresis slide, and the slides were exposed to the photographic film for a longer time in van Furth's study. The observation that radiolabeled γM may be synthesized as early as 10.5 wk gestation is interesting in view of the fact that lymphoid cells first become distinguishable in human lymphoid tissue at 8–9 wk gestation (32). Thus, differentiation into cells capable of producing γM occurs very early indeed in the development of the lymphoid cell system.

At approximately 3 wk gestation, the human liver can be distinguished as a thickened area of cells along the entodermal canal just caudal to the heart (13, 31). By 4 wk gestation, the human embryo has a crown-rump length of 4–5 mm, and the liver is a diverticulum of hepatic buds and ducts. At 4.5–5 wk gestation, when the embryo is approximately 10–15 mm in crown-rump length, hepatic lobes become recognizable (35). In the present study, the earliest stage at which the liver was cultured separately was 32 days gestation: this culture yielded radioactive β_{1C}/β_{1A}, C'1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, α_1-antitrypsin, orosomucoid, β-lipoprotein, α_2-macroglobulin, and prealbumin; additional studies also revealed the presence of radioactive albumin and α-fetoprotein in this culture. Except for ceruloplasmin and orosomucoid, each of these proteins was found in the culture of the whole 6.4 mm embryo of 29 days gestation. Thus, the human liver apparently can synthesize many different plasma proteins at a time when the hepatic cells are just appearing. On the other hand, the earliest that radioactive fibrinogen was detected was at 5.5 wk gestation. It is possible that fibrinogen had been synthesized in the earlier stages and had been precipitated in the cultures by clotting, in which case it would not have been detected, but this is unlikely in view of the fact that all liver cultures of 8.5 wk gestation or more yielded radioactive fibrinogen. In accord with the relatively late onset of fibrinogen synthesis in the human conceptus, we have observed in the past that blood from embryos of less than 6–8 wk gestation frequently failed to clot.

As has been reported by others (5, 36–41), synthesis of radiolabeled β_{1C}/β_{1A} was found in cultures of many different tissues. The macrophage or fibroblast appears to be at least one site of β_{1C}/β_{1A} synthesis in tissue culture and the hepatic cell another (39). In an orthotopic transplant of human liver, Alper and his coworkers (42) observed a conversion of the recipient's β_{1C} allotype to that of the donor. Since it was possible to miss detection of a small amount of the recipient's β_{1C} allotype in the presence of large amounts of the donor's allotype by the methods used, Alper and his colleagues were cautious to suggest that the liver may be the primary, but not necessarily exclusive, site of β_{1C} synthesis in vivo. The fact that relatively large amounts of radiolabeled β_{1C}/β_{1A} have been found in cultures of tissues besides the liver may be attributable to the obvious capacity of the fibroblast to grow in tissue culture much more readily than the hepatic cell. Interestingly, synthesis of C'1 esterase inhibitor was found to occur in most, but not all, of those tissues in which β_{1C}/β_{1A} was produced.

The serum ceruloplasmin concentration in some infants clearly decreased during the neonatal period, in two infants with a half-life of approximately 10 days; the fall was slower than the normal ceruloplasmin half-life of approximately 5–7 days (43, 33), suggesting that at least some of the postnatal ceruloplasmin was of fetal and neonatal origin. On the other hand, neonatal levels of transferrin were sustained postpartum, as found by Hitzig (8), indicating that the amount of transferrin synthesized by the neonate was equal to the amount being degraded. The neonatal concentrations of β_{1C}/β_{1A}, hemopexin, α_1-antitrypsin, β-lipoprotein, and α_2-macroglobulin postpartum indicated significant neonatal synthesis of these proteins as well.

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