THE ORIGIN OF PLASMA PROTEINS IN THE GUINEA PIG FETUS [1,2]

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During intrauterine life the fetus receives generous supplies of nitrogen from the mother for the formation of tissue proteins. In Figure 1 are presented diagrammatically the theoretical precursors of fetal nitrogen, stressing the origin of fetal plasma proteins. Omitted from consideration are the simpler nitrogenous compounds such as urea and ammonia which are not likely to be important sources of nitrogen for tissue protein in the presence of adequate supplies of amino acids. The possible pathways (Figure 1, reading from bottom up) are as follows:

1. Circulating amino acids in the mother may be transferred across the placenta and synthesized into proteins by the fetus.

2. Circulating amino acids in the mother may be synthesized into plasma proteins by the placenta for use by the fetus.

3. Maternal plasma proteins may be degraded by the placenta and resynthesized by the fetus.

4. Maternal plasma proteins may cross the placenta intact.

Some evidence concerning routes 1 and 2 in the human have been previously presented (1). In this paper will be presented the results of an investigation into routes 1, 3 and 4. The guinea pig was chosen as the experimental animal because, similar to the human, antibodies are transferred from mother to fetus in high titer (2). It was therefore hoped that results concerning other plasma proteins would also be applicable to the human.[4] The data we have obtained in the present study with isotopically labeled materials indicate that maternal amino acids and maternal plasma proteins serve as significant precursors of fetal plasma proteins. The placenta, however, does not degrade maternal plasma proteins for this purpose.

METHODS

Preparation of isotopically labeled materials. S^{35}-labeled plasma proteins were prepared biosynthetically by injecting 200 microcuries of S^{35} methionine,[5] intraperitoneally, into a guinea pig, and repeating the injection in eight hours. Twelve hours later the guinea pig was bled by cardiac puncture, using heparin as an anticoagulant. The plasma was separated by centrifuging and was dialyzed against four changes of 2 liters of isotonic saline over a 48 hour period at 4° C. S^{35}-labeled chicken plasma was prepared in a similar fashion from a donor chicken.

To obtain plasma protein fractions for injection, the radioactive plasma proteins were separated by zone electrophoresis on starch or on polyvinyl chloride resin [6] (3). The fractions were eluted with isotonic saline and then dialyzed against saline to free the material of veronal. As a rule, 4 to 5 ml. of plasma was separated on a block. Pooling of fractions from more than one block was usually not done in order to avoid additional manipulation
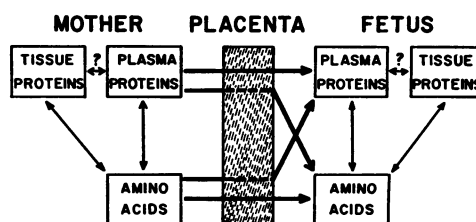

FIG. 1. DIAGRAMMATIC PRESENTATION OF POSSIBLE PRECURSORS OF FETAL PLASMA PROTEINS

pointed out that this may be a superficial similarity. In an interesting series of experiments, they have demonstrated that the route of transfer of antibody protein in the guinea pig is not across the placenta but is across the splanchnopleure, a route that is anatomically unavailable to the human.

and possibly damage of the proteins. If pooling was necessary, the pooled fractions were dialyzed against half-isotonic saline and then lyophilized to reduce to volumes that could be injected.

I^{131}-labeled plasma proteins were prepared according to the methods of Bauman, Rothschild, Yalow, and Berson (4) and of Pressman and Eisen (5).

Transfer experiments. Pregnant guinea pigs, estimated to be within two weeks of term, were used. Experiments were confined to this stage of pregnancy so that sufficient fetal blood could be obtained for analysis. The jugular vein of the mother was exposed surgically under Nembutal®-ether anesthesia and the injection made under direct vision. The amount of radioactivity injected varied with the material under study. For methionine, about 5 million counts per minute (cpm) with a specific activity of 20 million cpm per mg. was injected; total plasma, about 1 million cpm with a specific activity of about 20,000 cpm per mg.; plasma protein fractions, 100 to 300,000 cpm with the same specific activity. The fractions were in 15 ml. saline and were injected over a 20 minute period. Eighteen to 24 hours later a sample of maternal blood was obtained by cardiac puncture. The fetuses were delivered by hysterotomy and bled by decapitation.

In the experiments with radioiodinated proteins, the guinea pigs were given drinking water containing 0.3 N sodium iodide for 24 hours, and the injections were made with 2 ml. of 1.8 per cent sodium iodide in order to minimize utilization of liberated iodide. Seven $\times 10^6$ to

1.2×10^9 cpm of iodinated plasma proteins with specific activities ranging from 1.8×10^6 to 9×10^8 cpm per ml. was injected intravenously. The rest of the procedure was as described above.

Analytical methods. Maternal and fetal plasma proteins were separated by zone electrophoresis. The protein fractions were precipitated with equal volumes of 10 per cent trichloracetic acid (TCA) washed three times with 5 per cent TCA and redissolved in 0.05 N NaOH. An aliquot was taken for the determination of protein concentration by a modification of the method of Folin (6). For S^{35} determinations, the rest was transferred to planchettes. I^{131} radioactivity was determined in alkaline solution.

Determination of radioactivity. S^{35} radioactivity was determined in a D-46A Nuclear Flow Gas Counter, and the counts were corrected to infinite thinness. Many of the samples had little radioactivity. These were counted for one hour which provided a counting accuracy of at least plus or minus 10 per cent in those samples with observed counts of over 10 cpm over background. Those with less radioactivity were considered questionable and are indicated in the tables. I^{131} radioactivity was determined in a Tracerlab well-type scintillation counter, model SC 46.

RESULTS

Synthesis of plasma proteins by the guinea pig fetus

Guinea pig fetuses in the last week of gestation were delivered by hysterotomy and injected intraperitoneally with S^{35} methionine. Twenty-four hours later fetal blood was collected and the plasma proteins separated by zone electrophoresis. In Figure 2 are presented the protein curve and the radioactivity curve. There is no detectable incorporation of radioactivity into the gamma globulin fraction, although all other electrophoretic fractions appear to be synthesized by the fetus. This would indicate that the gamma globulin fraction is derived from either the placenta or the mother. Earlier investigations with human placenta (1) indicate that the placenta is an unlikely source.

The synthesis of plasma proteins by the fetus from amino acids transferred from the mother

S^{35} methionine was injected intravenously into pregnant guinea pigs and 24 hours later maternal and fetal blood samples were collected. Radioactivity was incorporated into all the plasma protein fractions in mother and fetus (Table I, A). The relatively high specific activities in the fetal plasma proteins are evidence of the rapid transfer of the amino acid and the efficient utilization by the

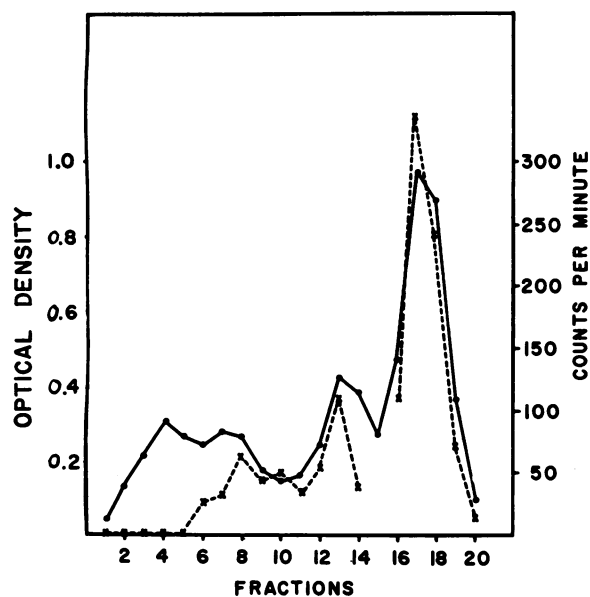FIG. 2. INCORPORATION OF S^{35} METHIONINE INTO FETAL PLASMA PROTEINS

$\times$ — — — $\times$, cpm per fraction; ● ———— ●, protein concentration per fraction [the observed optical density after developing color according to method of Folin (6)].

fetus. The notable exception is the fetal gamma globulin and the explanation probably lies in the inability of the fetus to synthesize this fraction. As a result the radioactivity must first be incorporated into maternal gamma globulin and then transferred to the fetus.

The transfer of S^{35}-labeled homologous plasma proteins across the placenta

The injection of S^{35}-labeled plasma proteins into the pregnant guinea pig resulted in the appearance of radioactivity in all the electrophoretic fractions in fetal plasma proteins (Table I, B). The results were very similar to those obtained by Whipple, Hill, Terry, Lucas, and Yuile in dogs with C^{14} lysine, although these investigators did not study the individual plasma protein fractions (7). The incorporation of radioactivity into fetal tissues in these experiments are not reported here but also agree well with those of Whipple and co-workers.

There still remains the possibility that the apparent transfer of intact plasma proteins actually represents degradation of the radioactive plasma proteins to smaller fragments which cross the placenta and are resynthesized by the fetus. This could be true of all fractions except the gamma globulin fraction. Although degradation of homologous plasma proteins by the placenta seems unlikely in view of the in vitro experiments reported below, this could result from maternal metabolism.

The transfer of heterologous plasma proteins labeled with S^{35} and I^{131}

The purpose of these experiments was to investigate the possibility indicated in the preceding paragraph. S^{35}-heterologous plasma proteins were used so that their fate could be followed both by radioactivity and by immunological methods. If the injected proteins were degraded and the radioactivity then incorporated into fetal plasma proteins, this would be readily detected immunologically.

S^{35} chicken plasma was injected into pregnant guinea pigs and 24 hours later maternal and fetal plasma were collected. Immunological identification of the radioactive proteins was made by preparing carrier precipitates in the plasmas with chicken plasma and an anti-chicken plasma (1) and then determining the amount of radioactivity incorporated in the specific precipitate (Table II). Most of this protein radioactivity (TCA-precipitable) in the maternal plasma was incorporated in the specific precipitate, but in the fetal plasma a considerable proportion was no longer identifiable immunologically as chicken plasma. Although these experiments were done with heterologous plasma proteins and might not be strictly applicable to those done with homologous plasma pro-

TABLE I

*Transfer of S^{35} methionine and plasma proteins from mother to fetus**

	Material injected	Maternal				Fetal			
		Albumin	Alpha	Beta	Gamma	Albumin	Alpha	Beta	Gamma
A	Methionine	161	297	367	156	59	217	183	20
		100	355	380	128	37	189	152	5
		146	312	436	230	63	225	190	26
B	Plasma proteins	81	80	100		9	13	11	25
		107	95	93	82	5	14	12	
		130	168	190	327	11	17	18	34
C	Albumin	240	15	3†	0	4	6	0	0
	Albumin	110	32	7	7†	4	3†	0	0
	Albumin	249	20	8†	4†	16	7	0	0
	Alpha	3	28	4	2	0	4	3†	0
	Beta-gamma	0	15	79	75	0	4†	2†	7
	Beta-gamma	0	6	46	135	0	0	21	17
	Gamma	0	0	29	59	0	0	0	6

* Specific activity in maternal and fetal plasma proteins in cpm per mg., 24 hours after injecting S^{35}-labeled materials into the mother.

† Calculated from less than 10 cpm above background.

TABLE II

Transfer of radioactive chicken plasma proteins across guinea pig placenta *

	Maternal		Fetal	
	TCA precipitate	Specific precipitate	TCA precipitate	Specific precipitate
S^{35} plasma proteins	14,850	10,820	950	150
	13,400	9,745	1,450	204
I^{131} plasma proteins	1,000,000	700,000	3,550	2,450
	1,100,000	965,000	4,000	3,850

* Twenty four hours after injection into the mother of the radioactive proteins, specific precipitates (chicken plasma plus anti-chicken plasma) were made in maternal and fetal plasma. Radioactivity incorporated in specific precipitates is compared with TCA-precipitable radioactivity (cpm per ml.).

teins, it was considered hazardous to interpret the results presented in the previous section as evidence for the transfer of intact plasma proteins.

Similar experiments with I^{131} chicken plasma produced significantly different results (Table II). Relatively less radioactivity was found in the fetal plasma protein, but the specific precipitate carried down the same proportion of the protein radioactivity in both maternal and fetal plasma. This indicated that the I^{131} proteins in the fetus were immunologically the same as those injected into the mother, and that the problem of degradation and reutilization would not confuse transfer experiments with this type of labeling. The results conform with the experience of investigators using isotopically-labeled plasma proteins for metabolic studies (8).

The transfer of S^{35}-labeled plasma protein fractions

In order to circumvent the problem of degradation and resynthesis and still use biosynthetically-labeled proteins, electrophoretically-separated fractions were injected. It was considered that if the pattern of radioactivity in the fetal plasma proteins differed from that obtained with S^{35} methionine, then the injected proteins were not degraded to the amino acid before reutilization. Evidence that the protein was transferred intact was obtained if the fetal fraction with the highest specific activity corresponded to that injected into the mother. The results are presented in Table I, C. Radioactivity was usually found in fractions adjacent to the one selected electrophoretically which we attributed to inaccuracies in the series of electrophoretic separations involved in preparation and analysis of samples.

When S^{35} methionine was injected into the

mother, the lowest specific activity was found in the fetal gamma globulin, intermediate in the albumin, and highest in the alpha and beta globulin fractions. In those experiments in which S^{35} albumin and gamma globulin were injected, the specific activity in the respective fetal fractions were equal to or exceeded that in the alpha and beta fractions. This was considered satisfactory evidence that these proteins had been transferred to the fetus intact. The data for the alpha and beta globulins is not inconsistent with the same conclusion but is inadequate. The distribution of radioactivity in these experiments is not sufficiently different from that obtained with methionine.

Only a small fraction of the total number of experiments (60) done with S^{35}-labeled plasma proteins is presented in Table I. The reasons for failure were multiple: abortions, inadequate fetal plasma samples, insufficient radioactivity in the fetal samples, and a pattern of radioactivity in the fetal samples that could not be differentiated from that expected with degradation of maternal proteins and resynthesis by the fetus. In none of the rejected experiments did the data suggest transfer of other than the injected fraction. In one experiment alpha globulin was injected into the mother and the specific activity of the fetal gamma globulin greatly exceeded the maternal. It was evident that radioactive contamination had occurred and the results were discarded.

The transfer of I^{131}-labeled plasma proteins

I^{131}-labeled plasma proteins were resorted to so that smaller amounts of high activity protein could be used, and to further exclude the possibility of degradation and resynthesis. The experiments

TABLE III

Degradation of plasma proteins by tissue slices *

Tissue	TCA precipitate	TCA-soluble
1. Maternal liver	21,000	170
Fetal liver	21,000	240
Placenta	21,000	230
2. Maternal liver	20,000	114
Fetal liver	20,000	97
Placenta	20,000	83

* Radioactive plasma proteins (TCA-precipitable radio-activity) were incubated with tissue slices for four hours and the amount of degradation (TCA-soluble radioactivity) was measured.

with I^{131} chicken plasma (Table II) had already given immunological evidence that the latter did not occur in the course of transfer of radioactivity to the fetus. It is well recognized that iodination of proteins produces some alteration in the protein but useful metabolic information has been obtained from the technique (8). It seemed a reasonable assumption that qualitatively reliable results would be obtained.

The first iodination method used (4) extensively degraded the alpha and beta globulin fractions. The injection of plasma iodinated by this method yielded radioactivity in the albumin and gamma globulin fractions in mother and fetus (Figure 3). The almost exact reduplication of the maternal pattern of radioactivity in the fetus gave electrophoretic confirmation (in addition to the immunological evidence cited above) that I^{131} proteins were transferred intact, and that degradation with reutilization did not occur. Using the same iodination procedure, Rothschild and Oratz (9) have demonstrated the transfer of I^{131} albumin in the rabbit. When organic solvents were avoided in the iodination procedure (5) significant radioactivity was found in all plasma protein fractions in mother and fetus (Figure 4). There were differences in the amount of radioactivity in the individual fetal electrophoretic fractions, but it is not possible to conclude that this reflects differences in the efficiency of transfer. The very low activity in the fetal alpha globulins may reflect poor transfer, but it may merely indicate that this fraction is altered more extensively during iodination. It may be pertinent to this problem that I^{131} thyroxine which is bound to, and in effect labels, the alpha globulins without artificial iodination of the
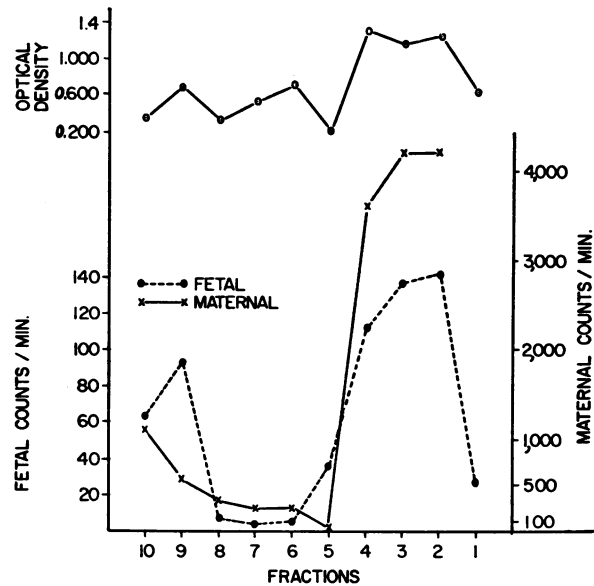

FIG. 3. TRANSFER OF I^{131} PLASMA PROTEINS ACROSS GUINEA PIG PLACENTA

The method of iodination in this experiment (4) apparently degraded the alpha and beta globulins. The top curve indicates protein concentration (6).

protein, is transferred in the rabbit from mother to fetus with about the same efficiency as albumin in the present study (10).

Degradation of maternal plasma proteins by placenta to provide precursors for fetal utilization

This possibility was investigated *in vitro* by incubating placental slices with S^{35}-labeled homologous plasma proteins. The extent of degradation was estimated by measuring the conversion of TCA-precipitable to TCA-soluble radioactivity. Slices of maternal and fetal liver were used as controls. The function of the liver is primarily synthetic (11).

Maternal and fetal liver, and placenta were obtained from pregnant guinea pigs under Nembutal®-ether anesthesia and dropped into chilled isotonic saline. Slices were prepared with a Stadie-Riggs slicer. The placental slices included both maternal and fetal surfaces. One gram of tissue was added to 2.5 ml. of Krebs-Ringer bicarbonate buffer (12) containing 10 μg. Aureomycin®, 5 mg. methionine, and radioactive plasma proteins in an amount sufficient to provide 20,000 cpm (3 to 6 mg.). The slices were incubated for four hours with constant shaking in a water
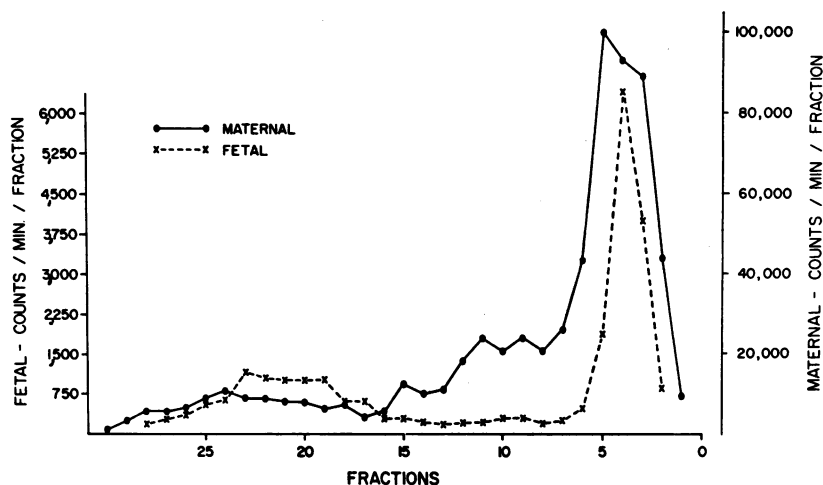
FIG. 4. TRANSFER OF I¹³¹ PLASMA PROTEINS ACROSS GUINEA PIG PLACENTA
Iodination of proteins as described by Pressman and Eisen (5).

bath at 37° C. At the end of incubation, the tissue slices were centrifuged down and discarded. Thirty-four mg. of methionine was added to the incubation medium and the proteins were precipitated with equal volumes of 10 per cent TCA. The supernatant was combusted and precipitated as barium sulfate, an aliquot of which was transfered to planchettes for determination of radioactivity (13). It is apparent from the table that the placenta does not demonstrate any specific capacity for the degradation of plasma proteins (Table III).

DISCUSSION

It is evident that maternal amino acids are transferred to the fetus where they are synthesized into plasma proteins. The fetal plasma proteins except for gamma globulin probably originate primarily in this way in the stage of pregnancy under investigation. The human fetal liver synthesizes plasma proteins efficiently as early as the third month of gestation (1) so that it is not unlikely that this is a major source of plasma proteins during most of pregnancy.

Evidence has also been presented for the transfer of maternal plasma proteins to the fetal circulation. The crucial experiments with S³⁵-labeled plasma proteins were those in which individual electrophoretic fractions were injected into the mother. It was only in this manner that the transfer of the intact protein could be differentiated

from the transfer of radioactive fragments to the fetus with subsequent reincorporation into proteins. This approach proved technically difficult so that relatively few of the completed experiments were considered sufficiently satisfactory for presentation (Table I,C). Although the radioactivity in the fetal plasma in these experiments was low, it was sufficient to be determined with reasonable accuracy (an observed radioactivity of at least one and one-half times background). It is noteworthy that radioactivity of this magnitude was never found in a fetal fraction other than the one injected into the mother, or the immediately adjacent electrophoretic fraction. This was further indication that the observation was significant.

With I¹³¹ plasma proteins the two major disadvantages of the biosynthetically prepared plasma proteins were avoided. It was possible to inject trace amounts of plasma proteins of high specific activity, and the interpretation of results was not confused by reutilization of radioactive fragments. Extensive experience in metabolic experiments has demonstrated that metabolites containing the iodine label are not reincorporated into proteins (8). We have presented immunological and electrophoretic evidence that this is also true under the conditions of our experiments. On the other hand, the major advantage of the biosynthetically-labeled plasma proteins, that of close identity with the native proteins, can only be approximated with the radioiodinated proteins. Close approximation

can be achieved in metabolic experiments, but the same information is not available relative to permeability. Thus the results with I^{131} may be considered complementary to those with S^{35} plasma proteins. These have been presented in detail in the previous section. The conclusion from both series of experiments is that maternal plasma proteins are transferred intact to the fetus in relatively large amounts.

The question arises as to how much of the fetal requirements for plasma proteins could be met by transfer of plasma proteins from the mother. Two factors must be evaluated in the answer: the efficiency of transfer and the metabolic requirements of the fetus. The metabolic half-lives of different plasma proteins vary widely in the human, from a matter of hours for antihemophiliac globulin to weeks for albumin. If this also pertains to the guinea pig fetus it would require considerably different rates of transfer to satisfy the fetal demands for these two materials. There is no information concerning the rates of utilization of plasma proteins by the fetus. Another feature which limits the interpretation is the heterogeneity of the electrophoretic fractions that were studied (14). This could hide significant differences in transfer among proteins within a fraction. Certain general conclusions can be safely drawn. In agreement with previous work, gamma globulin appears to be derived entirely from maternal plasma. Using this as a basis of comparison, significant amounts of fetal albumin and beta globulin are exchanged with maternal plasma proteins. The data relative to the alpha globulins is inconclusive.

SUMMARY

1. The guinea pig fetus near term can synthesize all plasma proteins recognizable electrophoretically except for gamma globulin.

2. The fetus efficiently utilizes amino acids transferred from the maternal circulation for the synthesis of plasma proteins.

3. *In vitro* studies failed to demonstrate any specific proteolytic powers in the placenta which degrade maternal plasma proteins to make available for the fetus precursors for plasma protein synthesis.

4. The transfer of maternal plasma proteins to the fetus was studied using radioactive plasma proteins prepared biosynthetically (S^{35}) and *in vitro* (I^{131}). The problems in interpretation of data with each type of radioactive labeling are discussed. It was concluded that other plasma proteins in addition to the gamma globulins are transferred in large amounts to the fetus.

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