On the Mechanisms of Maternofetal Transfer of Human Albumin and γG Globulin in the Mouse

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ABSTRACT Human serum albumin and human γG globulin were labeled with ^{131}I, and the labeled proteins were then mixed with different amounts of the respective unlabeled protein. These mixtures were injected intravenously into pregnant mice near term, and the amounts of protein-bound radioactivity present in the fetuses and in maternal serum 24 hr later were determined.

The concentration of human albumin found in the fetus was proportional to the maternal serum concentration of this protein over the maternal range studied, from 0.03 to 935 mg/100 ml. On the other hand, the fetal concentration of human γG first increased rapidly as the maternal concentration increased to approximately 200 mg/100 ml and then decreased as the maternal concentration continued to increase above this level; however, as the maternal human γG level increased above approximately 1100 mg/100 ml, the fetal concentration again increased and became proportional to the maternal concentration. The data suggest that maternofetal transfer of human γG in the mouse may be mediated by two processess; one of these, as with the transfer of human albumin, appears to be first order in relation to the maternal serum concentration, and the other appears to be consistent with a carrier or enzymatic process that is directly or indirectly inhibited at high maternal serum levels.

INTRODUCTION

The transfer of plasma proteins across the maternofetal barrier is, at least in part, independent of the molecular weight of the protein (1–3): γG globulin which has a molecular weight of 165,000, for example, traverses the human placenta much more readily than albumin which has a molecular weight of 65,000 (2, 3) or orosomucoid which has a molecular weight of 44,000 (3), and the latter proteins pass from mother to fetus more readily than does growth hormone which has a molecular weight of approximately 40,000 (4). This selectivity in maternofetal transfer has been attributed by Brambell (5) to a nonselective absorption of proteins on the maternal side of the barrier through pinocytosis, selective binding of pinocytosed proteins to specific receptors with protection of the bound protein from intracellular digestion, and subsequent release of the protected protein into the fetal circulation. The hypothesis requires that each receptor is relatively specific for a given protein, that the relative number of receptors for a given protein determines the relative amount of that protein to be transferred to the fetus, and that pinocytosed protein in excess of available receptors is catabolized.

In the present report, the net maternofetal transfer of human albumin and human γG at different maternal serum concentrations of these proteins was studied in the mouse because in that animal human γG is readily transferred from mother to fetus (6). Human albumin was selected because the maternofetal transfer of this protein in the mouse appeared to be much less efficient than that of human γG (6).

METHODS

Plan of study. Pregnant Swiss-Webster albino mice at 17–19 days of gestation were placed on drinking water containing 250 mg of NaI and 1 g of sucrose per 100 ml. The mice were injected intravenously 24 hr later with either human serum albumin-^{131}I to which different

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amounts of unlabeled human albumin were added or human γG-^{131}I supplemented with different amounts of unlabeled human γG. Each mouse received 1–2 μc of radioactivity, but the amount of albumin injected into each animal varied from 0.01 to 375 mg, and the amount of γG given per animal varied from 0.01 to 307 mg. A given amount of human albumin or γG was given to a group of three or four animals. The minimum volume of the proteins injected was dictated in large part by the maximum concentration of unlabeled protein that was added to the trace-labeled protein to make up the injection mixture; for albumin the maximum concentration was 25 g/100 ml and for γG it was 16 g/100 ml, and thus, the maximum volumes of albumin and γG injected were 1.5 and 2.0 ml, respectively. The latter volumes were not inconsequential, since they equaled approximately a third to a half of the blood volume of the mouse. However, the proteins were injected slowly over a period of 2–3 min, and the mice displayed no obvious untoward reactions to any of the injections.

24 hr after the injection, the mice were anesthetized with ether and exsanguinated by section of the axillary blood vessels; the serum was separated from the blood thus collected, and an aliquot of serum was then precipitated with 10 ml of 10% trichloroacetic acid (TCA). The mouse fetuses were promptly removed and the placenta was left in situ by clamping the umbilical cord and severing the cord below the clamp; there were from 9 to 15 fetuses per litter. The fetuses of a given litter were weighed and then homogenized in 10% TCA using a Waring blendor; the final volume of TCA homogenate was adjusted to 200 ml. The TCA precipitate of maternal mouse serum and duplicate 20-ml aliquots of the TCA fetal homogenate were centrifuged in plastic counting vials, and the precipitates were washed twice with 10% TCA. The radioactivity in each precipitate was estimated with a 3 inch well-type NaI crystal in conjunction with a RIDL 400 channel spectrometer; counting standards were used to avoid problems incident to radioactive decay, changes in the counting efficiency of the system, and differences in the geometry of the samples.

Labeled proteins. The human albumin that was radioiodinated had been obtained from pooled adult plasma by low temperature, ethanol-water fractionation; it was a twice crystallized albumin, preparation decanol-10, which has been characterized physicochemically and immunochemically in previous reports (7, 8). The γG which was iodinated was obtained from pooled normal human plasma by ethanol-water fractionation; it had a sedimentation coefficient of 6.8S in a 1% solution, and over 98% of the protein migrated as γG globulin on paper and cellulose acetate electrophoresis. The proteins were labeled with ^{131}I by a modification of the nitrous acid method of Pressman and Eisen (9) as described elsewhere (10). The efficiency of iodination was approximately 20%, and the labeled protein contained an average of approximately 0.5 atoms of iodine per molecule of protein. After removal of nonprotein radioactivity by passage through 1 cm by 5-cm columns of Dowex-2 resin followed by dialysis against several changes of 0.15 M NaCl for 24 hr, over 98% of the radioactivity of the iodinated protein preparations was precipitable with specific rabbit antiserum against the unlabeled protein, and over 99% of the radioactivity was precipitable in 10% TCA.

Carrier proteins. The unlabeled human serum albumin that was added to the radioiodinated albumin to provide different amounts of albumin for injection was obtained from Merck Sharp & Dohme (Lot No. 1737H). The unlabeled human serum γG used to supplement radioiodinated γG in the injection mixture was obtained from E. R. Squibb & Sons (Lot No. 367). Both proteins had been prepared by ethanol-water fractionation. The amounts of albumin and γG, respectively, in these preparations were determined immunochemically (11): the albumin contained no detectable γG, and approximately 2% of the protein in the γG preparation was albumin.

The concentrations of human albumin and human γG per milliliter of maternal mouse serum and per gram of fetal mouse were estimated from the amount of radioactivity for the respective protein per milliliter of serum and per gram of fetus divided by the amount of radioactivity per milligram of albumin or γG present in the injected solution. This calculation presumes, of course, that the unlabeled protein in the injection was metabolized at the same rate and behaved in the same way in the mouse as the labeled protein (12–14). The total weight of the fetuses in a given litter varied from litter to litter, because of differences in the number of fetuses per litter and differences between litters in the weight of the individual fetus. For these reasons, the fetal data are presented in terms of the amount of protein found per unit weight of fetus rather than the amount transferred per litter.

RESULTS

The relationship between the amount of human albumin (HA) or human γG (HG) injected and the maternal serum concentration that was found 24 hr after the injection is shown in Fig. 1. For equivalent amounts of protein injected, the maternal serum concentrations were higher for HG than for HA as would be expected, because of the differences in the half-lives of the two proteins.

The concentration of HA found in the mouse fetus 24 hr after injection of HA into the mother was proportional to the maternal serum HA concentration present at the same time (Fig. 2) and could be described by $F = 4.25\ M \times 10^{-3}$ ml/g, where F and M are the fetal tissue and maternal serum concentrations in mg/g and mg/ml, respectively. Since the maternal serum HA concentration varied linearly with the amount of HA injected (Fig. 1), the fetal tissue HA concentration was found to be proportional to the amount of HA injected as well. Thus, the ratio of the fetal HA

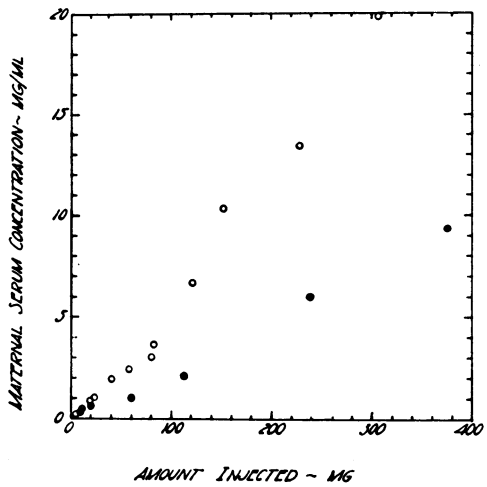

FIGURE 1 The maternal serum concentrations of human γG and human albumin found 24 hr after the intravenous injection of different amounts of these proteins. In this and in the subsequent figures, the open circles represent the values for human γG and the closed circles represent those for human albumin; each circle is the average of three to four pregnant mice.

concentration to the maternal serum HA concentration was observed to be constant over the entire range of the amounts of HA injected (Fig. 3), from 0.01 to 375 mg, and over the entire range of maternal serum HA concentrations present at 24 hr (Fig. 4), from 0.03 to 935 mg/100 ml.

On the other hand, the fetal: maternal HG ratio decreased rapidly as the maternal serum HG concentration increased (Fig. 4); the rate of decline decreasing gradually and the ratio becoming constant at maternal serum levels above 1100 mg/100 ml. When the fetal: maternal HG ratios were plotted against the amount of HG injected into the mother (Fig. 3), an identical fall in this ratio was found as the amount of Hg injected increased, the ratio becoming constant with injections containing more than approximately 200 mg of HG (Fig. 3).

Although the observed fetal HG concentrations appeared to be quite variable with increasing maternal HG levels (Fig. 2), connection of each plotted measurement circle to circle indicated that fetal HG concentration first increased rapidly, then declined, and finally increased again as maternal HG was increased. Upon transposing the smoothed curve (solid line) for the fetal: maternal HG ratios of Fig. 4 into Fig. 2 by simply multiplying the ratios delineated by the curve with the respective maternal HG concentrations, the curve shown as

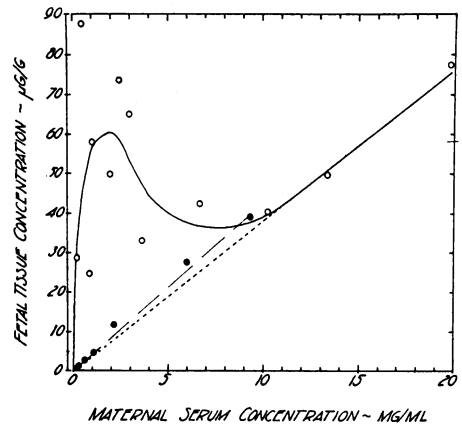

FIGURE 2 The concentration of human γG and human albumin in the fetus found at different maternal serum concentrations of these proteins 24 hr after intravenous injection into the mother. The solid line for the fetal HG concentrations was calculated from the smoothed curve (solid line) for fetal: maternal HG ratios shown in Fig. 4; the short dashed line is an extrapolation to zero of the first order relation between fetal and maternal concentrations of human γG found at higher maternal concentrations. In this and in the subsequent figures, each circle is the average for the litters from three to four pregnant mice.

the solid line in Fig. 2 was obtained, and the trend indicated by the individual fetal HG concentrations in Fig. 2 became more apparent. A rapid rise in fetal HG concentration as represented by the curve occurred as the maternal serum HG level was in-

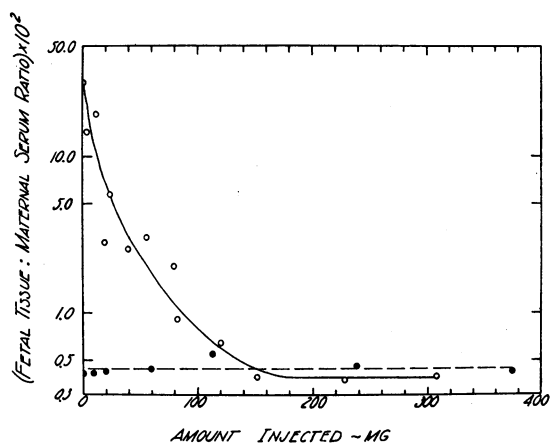

FIGURE 3 The ratio of the fetal concentration (milligram per gram) to the maternal serum concentration (milligram per milliliter) for human γG and human albumin 24 hr after intravenous injection of different amounts of these proteins into the mother. It should be noted that the ratios in this figure have been multiplied by a factor of 100.

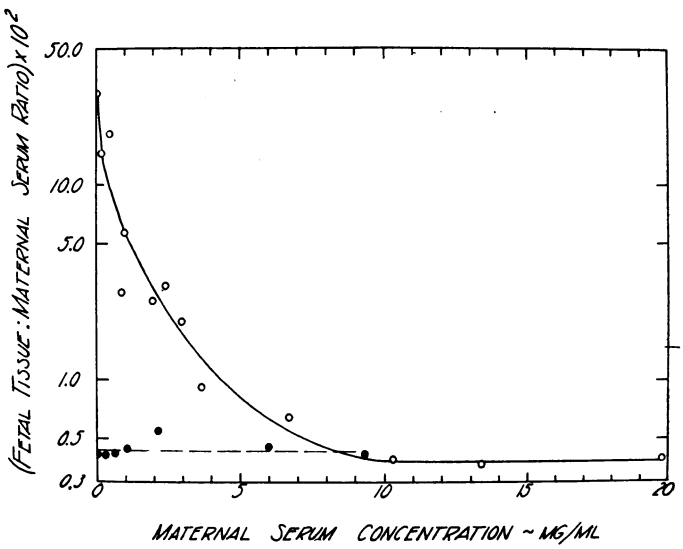

FIGURE 4 The fetal: maternal concentration ratio for human γG and human albumin found at different maternal serum concentrations of these proteins. As in Fig. 3, the ratios represented along the ordinate have been multiplied by a factor of 100.

creased to approximately 200 mg/100 ml, after which the fetal HG concentration decreased as the maternal serum HG level was increased to approximately 800 mg/100 ml. At maternal levels above approximately 1100 mg/100 ml, fetal HG appeared to be proportional to the maternal serum HG concentration and could be expressed by the relation $F = 3.80\ M \times 10^{-8}$ ml/g, where F and M are the fetal and maternal HG concentrations in mg/g and mg/ml, respectively.

Subtraction of the linear relation for HG, $F = 3.80\ M \times 10^{-8}$ ml/g, from the fetal tissue HG concentration curve in Fig. 2 yielded the curve in Fig. 5. Since the latter superficially resembled a carrier or enzymatic reaction inhibited by high substrate concentrations, the curve was replotted (15) as the reciprocal of the fetal tissue HG concentration, or $1/F$, vs. the reciprocal of the maternal serum HG concentration, or $1/M$. The resulting curve was a straight line when $1/M$ was equal to or greater than 1 ml/mg; extrapolation of this line gave 0.0145 g/μg as the $1/F$ intercept and − 3.25 ml/mg as the $1/M$ intercept. The maximum fetal tissue concentration that might be reached by this hypothetical system in the absence of inhibition during the 24 hr after the injection (15) is the reciprocal of the $1/F$ intercept, or 69 μg/g, and the equilibrium constant, K_s, for the dissociation of such HG-carrier or enzyme complexes would be the reciprocal of the $1/M$ intercept, or 0.31 mg of HG/ml. Reference to Fig. 5 will reveal that the maximum fetal HG on the curve is at a maternal serum HG level of approximately 1.5 mg/ml. At this point (15), the maternal HG concentration divided by K_s, or 1.5 mg/ml divided by 0.31 mg/ml, equals $(K_s'/K_s)^{\frac{1}{2}}$ where K_s' would be the equilibrium constant for the dissociation of a second or inhibiting molecule of HG from a hypothetical $(HG)_2$ carrier complex. Under these circumstances, $K_s' = (1.5/0.31)^2 K_s$, or 7.3 mg of HG/ml.

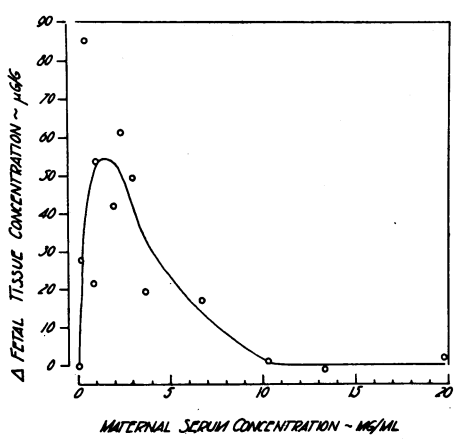

FIGURE 5 The curve for the relation between the fetal concentration and the maternal serum concentration of human γG obtained by subtraction of the first order relation between these concentrations from the individual circles and the solid line curve for human γG in Fig. 2.

DISCUSSION

The data suggest that the transfer of human γG from mother to fetus in the mouse may be mediated by two different mechanisms, one of which appears to be first order in relation to maternal se-

rum HG concentration, and the second appears to be a carrier or enzymatic process that is directly or indirectly inhibited at high maternal serum HG concentrations. At lower maternal serum HG levels, the hypothetical carrier or enzymatic process is the more efficient, but transfer by the first order process predominates at high maternal concentrations of HG. Since the ratio $K_s'/4K_s$ for the carrier or enzymatic process exceeds unity, it would seem that the affinity of the enzyme or carrier for the first molecule of HG is greater than that for subsequent molecules of HG (16).

The concentration of a given protein in the fetus after intravenous injection into the mother is dependent in part upon the rate of disappearance of the protein from the fetus as well as the concentration of protein in the maternal circulation and the rate of transfer of the protein across the maternofetal barrier (3). The disappearance of protein from the fetus is, of course, dependent in part upon the fetal rate of degradation of the protein. Therefore, it could be argued that the observed changes in the amount of human γG found in the fetus with increasing maternal concentrations might be attributable to changes in the fetal degradation of the protein rather than to specific changes in maternofetal transport. Such changes in fetal metabolism would have to be specific for human γG, since they are not reflected in the amounts of human albumin found in the fetus. Although increasing the concentration of human γG in mice does, within limits, specifically decrease the half-life of human γG (17), it should be noted (Fig. 2) that the fetal concentration of human γG rose then fell, and again rose as the maternal concentration was increased. Thus, the fetal concentrations of human γG were similar at widely different maternal concentrations of human γG; under these circumstances, the concentration effect of human γG on its own metabolism cannot account for the changes in fetal tissue concentration. It is almost unnecessary to state, however, that the possibility remains that there may exist some other, although unknown, specific alteration of fetal metabolism which may account for the observed changes in the fetal concentration of human γG.

Whether mouse γG is transferred by a substrate-inhibitable system similar to that for human γG or whether mouse γG competes with human γG for the same system cannot be determined from the data obtained in this study. However, when approximately 0.01 mg of radioiodinated mouse γG was injected into pregnant mice near term, the fetal tissue: maternal serum ratio for labeled protein in the presence of normal maternal serum concentrations of endogenous mouse γG was only 2.5×10^{-2} ml/g at 24 hr after injection (6). When 0.01 mg of labeled human γG was injected into the pregnant mouse, the fetal: maternal ratio in the presence of normal maternal mouse serum γG concentrations in this study was approximately 30×10^{-2} ml/g. This ratio was 12 times the ratio for labeled mouse γG under the same conditions, and hence 12 times the fetal: maternal ratio for endogenous maternal mouse serum γG that was transferred to the fetus during the 24 hr after injection. Thus, if mouse γG does compete with human γG for the same transport system, the degree of this competition would appear to be quite small at normal serum concentrations of mouse γG and low concentrations of human γG.

A selective transport system for the transfer of human γG from mother to fetus does exist in the human placenta (2, 3). Whether this system can be inhibited by high maternal serum γG concentrations is not known, but it has been noted by Edozien that infants born to mothers whose serum γG concentrations were below 1.6 g/100 ml tended to have serum γG levels higher than those of their mothers, and infants born to mothers whose serum γG levels were above 1.6 g/100 ml tended to have serum γG concentrations less than those of their mothers (18).

The net maternofetal transfer of human albumin in the mouse appears to be proportional to the maternal serum concentration. Such first order relationships occur in a number of processes like diffusion or, among others, in carrier or enzyme systems in which the carrier or enzyme occurs in large excess. It was noted that the proportionality constant for albumin transfer was 4.25×10^{-3} ml/g whereas that of the first order process for human γG transfer was 3.80×10^{-3} ml/g. The similarity in the proportionality constants for the two proteins suggest that diffusion might be the more likely process operating. A single hypothetical carrier system for both proteins would have to be nonspecific or else multiple carriers would have to be postulated, and, in either case, the carrier or carriers would have to have similar kinetics for the two proteins.

It should be noted that the data do not rule out

a specific substrate-inhibitable transport system for human albumin similar to that noted for human γG. If such a system did exist, it might not have been observed under the conditions of this study if mouse albumin competed effectively with human albumin. Under such circumstances, the effective maternal concentration during HA transfer would have been that for HA plus the effective mouse albumin concentration; the latter concentrations might have completely inhibited such a system, if such a system did exist at all, even when the transfer of trace amounts of human albumin was studied.

Brambell has attributed selectivity of maternofetal protein transport to specific cellular receptors, and hypothesized that there were fewer receptors for albumin than for γG (5). Under such circumstances, the amount of protein transferred from mother to fetus should increase as the maternal concentration of that protein increased until the amount transferred to the fetus reached a maximum constant value that would be proportional to the number of specific receptors. This situation was not observed for either human albumin or for human γG in this study. If the first order processes for human albumin and human γG transfer that were observed here are to be attributed to specific receptors, there was no evidence of saturation of such receptors, and since the proportionality constants for the two proteins were similar, the process would hardly serve as the basis for selective transport. Selective transport of human γG under the conditions of this study seemed to be consistent with a specific inhibitable carrier or enzyme system. If a carrier or receptor system exists for human albumin at lower mouse albumin concentrations, it, too, would have to be an inhibitable system, since no evidence of it was observed at normal mouse albumin concentrations.

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