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amino isobutyric acid
fetal growth
methylglucose
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placental blood flow

Placental Blood Flow and Transfer of Nutrient Analogs in Large, Average, and Small Guinea Pig Littermates

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Summary

Placental blood flow in the maternal side and transfer of [^{14}C]-α-amino isobutyric acid (AIB) and [^{3}H]methylglucose (MG) were measured in 22 pregnant guinea pigs at various gestational ages. The fetuses were divided in three groups according to their body weights: small, average, and large. Body weight was 85.25% of average values in the small fetuses and 114.12% in the large fetuses. Placental weight was 121.73% of average in the large fetuses and 84.42% in the small fetuses. Placental blood flow was 134.48% of average in large fetuses and 73.18% in small fetuses. AIB and MG transfers were significantly lower in the small fetuses (80.33% and 86.06%, respectively, of average values). In contrast, in large fetuses, AIB transfer was 123.43%, and MG transfer was 113.96% of average. Significant correlations were found between fetal and placental weight and placental blood flow and transfer of AIB and MG in the various groups. Placental transfer of AIB and placental blood flow were significantly correlated in the small (r = 0.59) and average weight fetuses (r = 0.37). In addition, the slope of the regression curve for AIB was significantly steeper in the small fetuses when compared with the slope of average and large fetuses. Placental blood flow and transfer of MG were significantly correlated only in the large fetuses (r = 0.48). In the small fetuses, the rate of AIB transfer was proportionally more reduced than that of MG transfer as the rate of placental blood flow decreased.

The results demonstrate that spontaneous fetal growth retardation in the guinea pig is associated with a smaller placenta, a reduced placental blood flow, and a reduced transfer of AIB and MG. Inasmuch as in the small fetuses AIB transfer was proportionally more reduced than MG transfer, it is suggested that in addition to the reduced blood flow limited availability of certain essential amino acids may be a cause of fetal growth retardation.

Speculation

A reduced placental transfer of certain essential amino acids may further aggravate the fetal growth retardation associated with low placental blood flow.

Fetal growth is influenced by the interaction of intrinsic or genetic factors and extrinsic factors imposed by the intrauterine environment. In turn, the intrauterine environment is influenced by such maternal factors as size, parity, smoking, and nutritional status (4). The mechanisms by which these maternal factors influence fetal growth remain unknown.

In polytocous animals such as the mouse, the rat, and the guinea pig, the rate of placental blood flow is believed to be the major determinant of fetal growth. For instance, the inverse correlation between mean fetal weight and the number of fetuses in one uterine horn has been attributed to reduced placental blood perfusion (8). Similarly, the smaller size of the fetuses in certain positions within the uterine horns also has been attributed to hemodynamic factors (8). The relation of fetal growth to uterine blood flow is further supported by experiments demonstrating that a chronic reduction in uterine blood flow causes various degrees of fetal growth retardation in the rat and sheep (2, 11). Moreover, recent studies suggest that reduced placental blood flow (in the maternal side) mediates the growth-retarding effect of maternal malnutrition in the rat (6). Thus, reduced placental blood perfusion seems to be the major cause of fetal growth retardation in animals under a variety of experimental conditions. However, maternal placental blood flow in cases of "spontaneous" fetal growth retardation has not yet been measured to test this hypothesis.

This report presents the results of a study in which placental blood flow in the maternal side and placental transfer of non-metabolizable nutrient analogs were studied in fetal guinea pigs of different sizes although of similar gestational ages carried by the same mother.

MATERIALS AND METHODS

Pregnant guinea pigs (Hartley strain) aged 6 to 8 months kept under standard laboratory conditions were anesthetized with ketamine hydrochloride (Vetalar, 20 mg/kg SC) and immobilized over a heated cushion. A tracheostomy was performed, the right jugular vein was catheterized, and pentobarbital (6 mg/kg) was injected IV. After the pentobarbital injection, a lactate-Ringer solution was infused at a constant rate (3 ml/min) throughout the same vein during the experiment. A catheter was inserted into the left carotid artery, and the tip was positioned in the left ventricle (this was verified at the end of each experiment). The right saphenous or femoral artery was also catheterized and connected to a Statham P 23 AC transducer for continuous monitoring of blood pressure. The left saphenous or femoral artery was similarly catheterized and connected to a withdrawing pump (Sage Instruments model 351) for blood sampling. Rectal temperature was continuously monitored by a telethermometer (Yellow Springs Instrument Co. model 43 TA), and kept constant at 38.5°C. Immediately after the experimental period, blood was sampled from the left femoral artery for hematocrit and blood gas determinations.

At time 0 of the experimental period, [^{14}C]α-amino-isobutyric acid (AIB) (10 μCi/kg) (51.63 mCi/mmole) and [^{3}H]methylglucose (MG) (100 μCi/kg) (80.8 Ci/mmole) were injected into the right jugular vein. Placental blood flow was determined using ^{51}Cr-labeled 25 μ diameter microspheres (3M Co., St. Paul, MN) (10 mCi/g). The microsphere was suspended in 10% dextran containing 0.05% Tween 80 surfactant. Before use, the microsphere suspension was sonicated for 10 min and then thoroughly mixed. Twenty-five min after the injection of AIB and MG, withdrawal of blood for the reference sample was begun using the femoral artery catheter. Withdrawal rate was 2.3 ml/min. A few sec later, 0.5 ml of the microsphere solution (approximately 450,000 spheres) was injected in 10 sec into the left ventricle (via the

carotid artery catheter). The catheter and the syringe used for the infusion were flushed using the animal's own blood. Withdrawal of blood continued for a total time of 2 min. In several animals, a second blood sample taken from the femoral artery at the end of the blood sample reference period revealed no circulating microspheres. Five min after the microspheres were injected, the animals were killed with an overdose of pentobarbital.

The reference blood sample was placed into a counting vial and weighed to determine the exact rate of withdrawal. A few drops of detergent were added to hemolyze the blood and facilitate sedimentation of the spheres. This ensured uniformity of the geometric effect in all counting vials. Individual fetuses and placentas were removed, and their positions in the uterus were recorded. The subplacenta, umbilical cord, and membranes were discarded. The main placenta was minced and transferred to a counting vial. ^{51}Cr radioactivity was determined in a gamma scintillation spectrometer (Packard model 3002). The approximate number of microspheres present in each suspension as well as the specific activity of the suspension injected in each experiment were determined by counting the number of microspheres present in a small aliquot. Determinations of the radioactivity of this sample made it possible to estimate the specific activity of the injected microspheres. Cardiac output and placental blood flow were determined according to the method of Rudolph and Heymann (10).

To determine the concentration of [^{14}C]AIB and [^{3}H]MG, fetuses and placentas were homogenized in four volumes of distilled water, and to 1.5 ml aliquots of the final homogenates, 0.5 ml of 20% trichloroacetic acid was added to precipitate proteins and cellular debris. The samples were then centrifuged for 10 min at 5000 × *g*. The supernatants were collected, and the pellets were washed with 1 ml of 5% trichloracetic acid and recentrifuged. Ten ml of Aquasol-2 (New England Nuclear, Boston, MA) were added to 1 ml of the combined supernatant, and the samples were counted in a liquid scintillation spectrometer (Packard Tricarb model 3255). The counting efficiency in the ^{14}C and ^{3}H channels was determined with standard quenching curves.

To compare results from animals of different gestational ages, all values were expressed as percentage of mean values for a given litter. Based on their body weight, the fetuses were divided in three groups: (1) fetuses whose body weight was reduced by 10% or more than the mean litter value (small fetuses); (2) fetuses whose body weight was increased 10% or more than the mean litter value (large fetuses); and (3) fetuses whose body weight was within ±9.9% of their respective mean litter value (average fe-

Table 1. *Selected maternal variables during the experiment (n = 22)*

Maternal weight (g)	1091 ± 26[1]
Systolic blood pressure (mm Hg)	70 ± 1
Diastolic blood pressure (mm Hg)	53 ± 1
Heart rate (beats/min)	218 ± 4
Arterial pH (n = 20)	7.43 ± 0.01[2]
Arterial Po_2 (n = 20)	80.6 ± 3.5[2]
Arterial Pco_2 (n = 20)	34.3 ± 1.3[2]
Hematocrit	35.5 ± 0.3
Cardiac output (ml/min)	201 ± 17
Cardiac output (ml/min/kg)	185 ± 16
Litter size	4.6 ± 0.2
Gestational age (days)	54 ± 2

[1] Mean ± S.E.

[2] Samples taken at the end of the experiment.

Table 2. *Fetal weight, placental weight, placental blood flow, and AIB and MG transfer in small, average, and large fetuses*[1]

	Large[2] (n = 24)	Average (n = 51)	Small[2] (n = 26)
Fetal weight	114.12 ± 0.91[3]	100.89 ± 0.71	85.25 ± 0.82
Placental weight	121.73 ± 2.77	97.72 ± 1.52	84.42 ± 2.08
Placental blood flow	134.48 ± 6.75	97.47 ± 4.25	73.18 ± 3.73
Total AIB transfer	123.43 ± 2.72	99.01 ± 1.55	80.33 ± 2.94
Total MG transfer	113.96 ± 1.33	100.53 ± 0.89	86.06 ± 0.97

[1] Values are expressed as a percentage of mean litter values.

[2] All values statistically different from average ($P < 0.001$).

[3] Mean ± S.E.

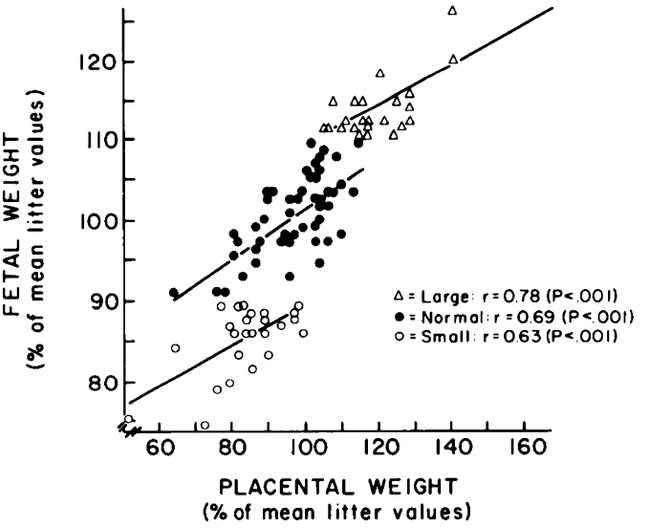

Fig. 1. Correlation between fetal and placental weights in small, average, and large fetuses. Values are expressed as percentage of mean litter value. The linear regression equations ± S.E. for the various groups are the following: large fetuses (n = 24): y = 82.93 + 0.26 x ± 2.82, r = 0.78 ($P < 0.001$); average fetuses (n = 51): y = 69.20 + 0.32 x ± 3.67, r = 0.69 ($P < 0.001$); small fetuses (n = 26): y = 64.20 + 0.25 x ± 3.27, r = 0.63 ($P < 0.001$).

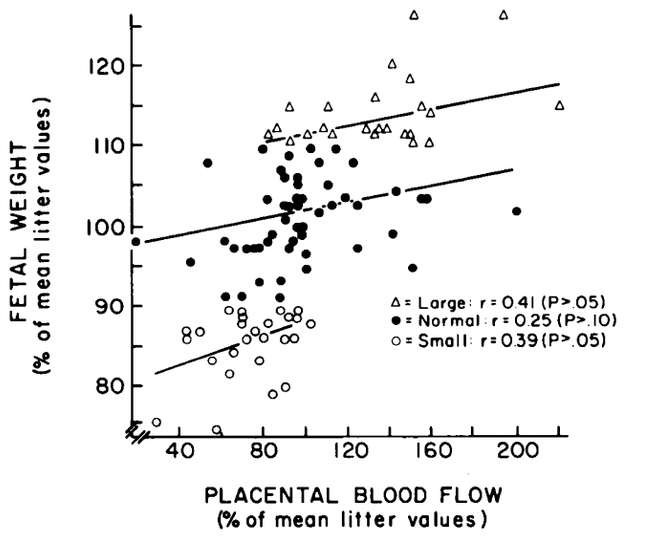

Fig. 2. Correlation between placental blood flow and fetal weight in small, average, and large fetuses. Values are expressed as percentage of mean litter value. The linear regression equations ± S.E. for the various groups are the following: large fetuses (n = 24): y = 106.70 + 0.05 x ± 4.13, r = 0.41 ($P > 0.05$); average fetuses (n = 51): y = 96.85 + 0.04 x ± 4.95, r = 0.25 ($P > 0.10$); small fetuses (n = 26): y = 78.93 + 0.09 x ± 3.89, r = 0.39 ($P > 0.05$).

tuses). For the final data analysis, only the litters in which these three groups were recognizable were included.

The statistical significance of differences in mean values for the various groups was determined using Student's t test. Simple linear regression equations were calculated for different variables for all fetuses and the different groups. The statistical significance of each regression coefficient was determined by analysis of variance. Differences in regression coefficient values among groups were determined with a Student's t test comparing population slopes (13).

RESULTS

Twenty-two pregnant guinea pigs carrying a total of 101 fetuses were studied between days 29 and 65 of pregnancy. As indicated by blood pressure, cardiac output, arterial P_{O_2} and P_{CO_2} values and other variables, the animals were in good metabolic and ventilatory conditions during the experiments (Table 1).

The mean body weight of the small fetuses was 85.25% of mean litter weight values whereas the mean body weight of the large fetuses was 114.12% of mean litter weight values. Placental weight, blood flow, and AIB and MG transfer were significantly lower than average in the small fetuses and significantly higher than average in the large fetuses (Table 2). Gestational age did not influence group differences. In both small and large fetuses, placental blood flow values deviated from average proportionally more than body weight values. Placental blood flow was 27% reduced in the small fetuses and 34% increased in the large ones. In small fetuses, total AIB transfer was proportionally more reduced than MG transfer, but this difference was not statistically significant. In contrast, the higher rate of AIB transfer in the large fetuses (123.43%) was proportionally and significantly bigger ($P < 0.005$) than MG transfer (113.96%).

A significant and similar correlation was found between fetal and placental weights in the various groups (Fig. 1). Similarly, fetal weight of all groups combined was significantly correlated with placental blood flow ($r = 0.64$; $P < 0.001$). However, because of considerable individual variability, fetal weight and placental blood flow were not significantly correlated in the different groups (Fig. 2).

Fetal weight and placental transfer of AIB were significantly correlated in all fetuses ($r = 0.86$; $P < 0.001$) and also in the various groups. Further, the slope of the curve was significantly less steep in the small fetuses when compared with average size fetuses ($P < 0.05$) (Fig. 3A). Fetal weight and placental transfer of MG were also significantly correlated in all fetuses ($r = 0.93$; $P < 0.001$) and individual groups (Fig. 3B). There was a significant

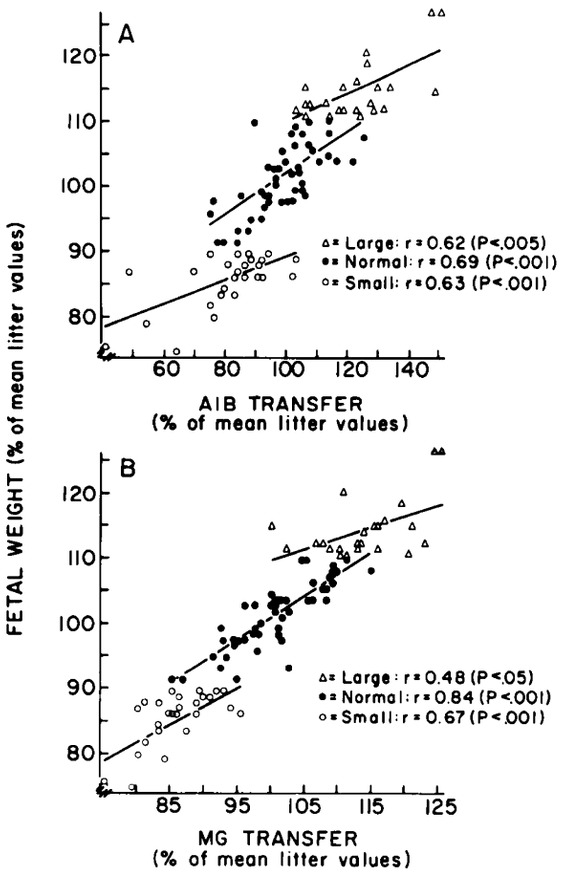

Fig. 3. Correlation between placental AIB (A) and MG (B) transfer and fetal weight in small, average, and large fetuses. Values are expressed as percent of mean litter value. For AIB transfer, the linear regression equations ± S.E. are the following : large fetuses ($n = 24$): $y = 88.69 + 0.21 x \pm 3.57$, $r = 0.62$ ($P < 0.005$); average fetuses ($n = 51$): $y = 69.52 + 0.32 x \pm 3.69$, $r = 0.69$ ($P < 0.001$); small fetuses ($n = 26$): $y = 71.12 + 0.18 x \pm 3.27$, $r = 0.63$ ($P < 0.001$). For MG transfer, the linear regression equations ± S.E. are the following: large fetuses ($n = 24$): $y = 76.54 + 0.33 x \pm 3.98$, $r = 0.48$ ($P < 0.05$); average fetuses ($n = 51$): $y = 33.65 + 0.67 x \pm 2.77$, $r = 0.84$ ($P < 0.001$); small fetuses ($n = 26$): $y = 36.60 + 0.56 x \pm 3.16$, $r = 0.67$ ($P < 0.001$).

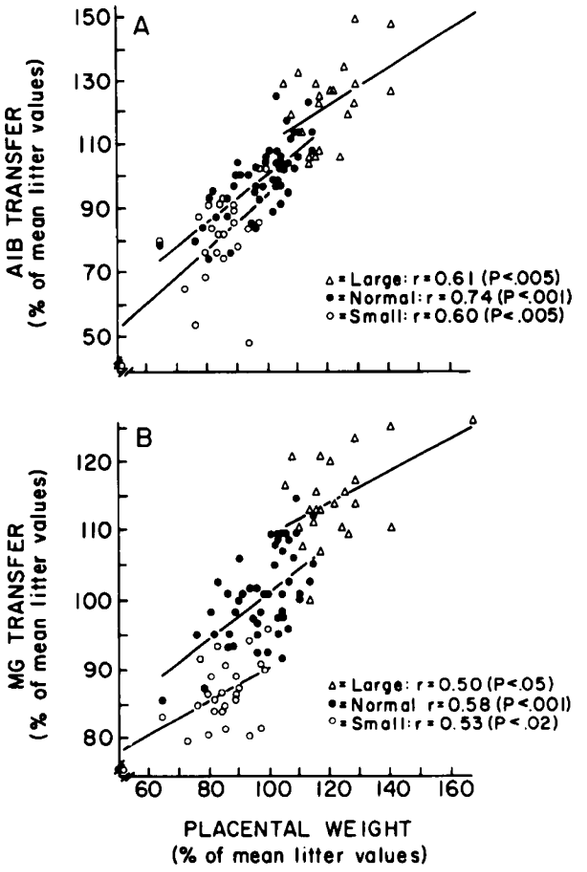

Fig. 4. Correlation between placental weight and AIB (A) and MG (B) transfer in small, average, and large fetuses. Values are expressed as percentage of mean litter value. For AIB transfer, the linear regression equations ± S.E. ar the following: large fetuses ($n = 24$): $y = 50.80 + 0.60 x \pm 10.79$, $r = 0.61$ ($P < 0.005$); average fetuses ($n = 51$): $y = 25.33 + 0.75 x \pm 7.51$, $r = 0.74$ ($P < 0.001$); small fetuses ($n = 26$): $y = 8.06 + 0.86 x \pm 12.20$, $r = 0.60$ ($P < 0.005$). For MG transfer, the linear regression equations ± S.E. are the following: large fetuses ($n = 24$): $y = 84.94 + 0.24 x \pm 5.73$, $r = 0.50$ ($P < 0.05$); average fetuses ($n = 51$): $y = 67.02 + 0.34 x \pm 5.21$, $r = 0.58$ ($P < 0.001$); small fetuses ($n = 26$): $y = 65.32 + 0.25 x \pm 4.24$, $r = 0.53$ ($P < 0.02$).

difference in the slope of the regression curves between large and average size fetuses ($P < 0.01$).

A significant correlation was also found between placental weight and transfer of both AIB and MG in all fetuses combined and also in the individual groups of fetuses (Fig. 4).

Placental weight was correlated with placental blood flow in all fetuses ($r = 0.65$; $P < 0.001$) and in small and average weight fetuses. No significant correlation between these two variables was found in the large fetuses (Fig. 5).

Placental transfer of AIB and placental blood flow were significantly correlated in all fetuses combined ($r = 0.67$; $P < 0.001$) and also in the small and average weight fetuses. In addition, the slope of the regression curve was significantly steeper in the small fetuses compared with the average weight fetuses ($P < 0.02$) (Fig. 6*A*). Placental transfer of MG and placental blood flow were significantly correlated only in all the fetuses combined ($r = 0.61$; $P < 0.001$) and in the large fetuses ($r = 0.48$; $P < 0.05$; Fig. 6*B*). A comparison between AIB and MG transfer in the small fetuses revealed that the slope of the correlation curve was significantly much steeper for AIB ($P < 0.01$), suggesting that when placental blood flow is markedly reduced AIB transfer is proportionally more affected than MG transfer (Fig. 6).

DISCUSSION

Present data demonstrate that in the guinea pig spontaneous fetal growth retardation is associated with a smaller placenta, a reduced placental blood flow, and a reduced transfer of AIB and MG into the fetus.

The results support the idea that fetal growth is largely dependent on the availability of nutrients and oxygen determined by the rate of placental blood flow (3, 12). The smaller placental size, which in the small fetuses is proportional to the fetal growth retardation, is probably also determined by the reduced placental blood flow. Thus, the type of fetal growth retardation present in the guinea pig model would be determined primarily by a reduced placental blood flow that, in turn, will determine placental and fetal growth retardation. The key aspect which still remains unsolved is, of course, the reason for a reduced placental blood flow in certain fetuses. In the mouse, the incidence of runting has been associated with the rate of blood perfusion in certain areas of the uterine horn (8). For example, the fetuses on both ends of the uterine horns, irrigated by the uterine and ovaric arteries, are larger than the fetuses irrigated by anastomotic branches of these two arteries. In the present study, however, the smaller fetuses were not distributed in any clear pattern.

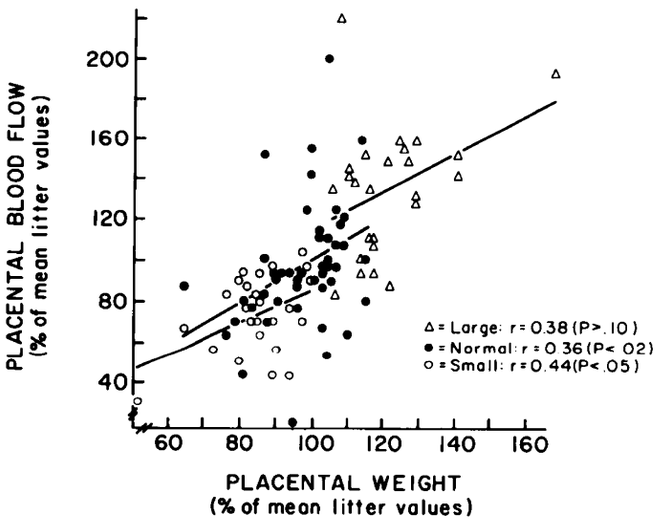

Fig. 5. Correlation between placental weight and placental blood flow in small, average, and large fetuses. Values are expressed as precent of mean litter value. The linear regression equations ± S.E. for the various groups are the following: large fetuses ($n = 24$): y = 20.56 + 0.94 x ± 31.20, $r = 0.38$ ($P > 0.10$); average fetuses ($n = 51$): y = −1.82 + 1.02 x ± 28.56, $r = 0.36$ ($P < 0.02$); small fetuses ($n = 26$): y = 6.12 + 0.79 x ± 17.41, $r = 0.44$ ($P < 0.05$).

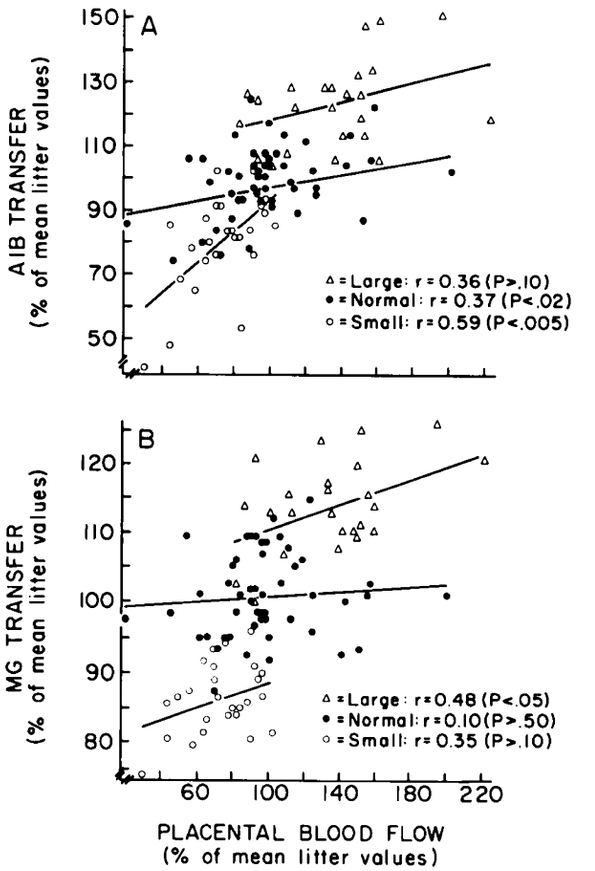

Fig. 6. Correlation between placental blood flow and AIB (*A*) and MG (*B*) transfer in small, average, and large fetuses. Values are expressed as percentage of mean litter value. For AIB transfer, the linear regression equations ± S.E. are the following: large fetuses ($n = 24$): y = 103.74 + 0.15 x ± 12.67, $r = 0.36$ ($P > 0.10$); average fetuses ($n = 51$): y = 85.88 + 0.13 x ± 10.37, $r = 0.37$ ($P < 0.02$); small fetuses ($n = 26$): y = 46.31 + 0.46 x ± 12.36, $r = 0.59$ ($P < 0.005$). For MG transfer, the linear regression equations ± S.E. are the following: large fetuses ($n = 24$): y = 101.41 + 0.09 x ± 5.82, $r = 0.48$ ($P < 0.05$); average fetuses ($n = 51$): y = 98.49 + 0.02 x ± 6.39, $r = 0.10$ ($P > 0.50$); small fetuses ($n = 26$): y = 79.47 + 0.09 x ± 4.69, $r = 0.35$ ($P > 0.10$).

An interesting finding of the present study is the marked reduction in AIB transfer in the fetuses where placental blood flow was the lowest. Because in these fetuses MG transfer was reduced proportionally to the reduction in body weight, the fetuses were receiving proportionally less AIB than MG. This finding suggests the possibility that a limited availability of certain amino acids may have a role in the etiology of fetal growth retardation.

The sensitivity of placental transfer of certain amino acids to the rate of placental blood flow has been demonstrated in the guinea pig(s). In an acute experiment in which uterine-placental blood flow was reduced 30% by reducing maternal blood volume, the placental transfer of glucose and α-amino nitrogen decreased 35% whereas transfer of leucine and lysine decreased by 60% (5). A similar disproportion in the rates of placental transfer of AIB and [^{3}H]2-deoxyglucose during conditions of reduced blood perfusion is apparent in a study performed in rats with uterine artery ligation (9).

There is a limited amount of information available on the plasma amino acid profile of growth-retarded newborns. Some of this information is hard to interpret because of the heterogeneity

of the sample classified as intrauterine growth retarded. It has been reported, however, that small-for-gestational age infants have an abnormal amino acid profile in plasma compatible with inadequate transfer of certain amino acids (7). In view of present results, this aspect of fetal growth retardation should be reexamined.

Although placental blood flow had a positive correlation with fetal weight when all fetuses were analyzed together, the correlation was nonsignificant for the various groups analyzed separately. This lack of correlation reflects a marked individual variation of placental blood flow values. Inasmuch as maternal conditions were uniform for all fetuses, the variability may reflect methodologic error inherent in the microsphere technique. This explanation seems unlikely, however, because simultaneous measurements of blood flow to maternal kidney and ovary carried out in the same animals (to be reported elsewhere) have a much smaller degree of variability. An alternative explanation is that in polytocous animals placental blood flows are constantly fluctuating over a basal rate of blood flow necessary to maintain adequate oxygenation. The alternating periods of higher placental blood flow may be under the control of either placental or fetal factors and would represent "feeding" periods for the various fetuses. Interestingly, determination of placental blood flow in the rabbit, also using microspheres, has shown a similar degree of marked differences in the individual fetuses (1).

Values of placental blood flow obtained in anesthetized animals are unlikely to reflect values present in a free-living unstressed guinea pig because of the expected changes in uterine blood flow. Nevertheless, there is no reason to suspect that possible reductions in uterine blood associated with anesthesia and surgical manipulations may alter the pattern of distribution of blood flow in the various placentas. Thus, although the absolute values may not correspond to those present in a nonanesthetized animal, it seems fair to assume that the relative differences present in fetuses of different size are real. The overall direction of the reported changes supports this contention.

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