Pediatr. Res. 16: 716–720 (1982)

Uteroplacental Blood Flow during Pregnancy in Chronically Catheterized Guinea Pigs

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Summary

Uterine blood flow and its distribution to uterus, placenta and subplacental structures was measured in 18 pregnant guinea pigs studied under chronic steady-state conditions. Maternal cardiac output averaged 266 ± 14 ml/min. Placental blood flow (PBF) remained less than 4 ml/min until 50 days gestation, after which it increased rapidly, attaining flows of 16 ml/min at term. PBF also increased linearly with placental weight ($r = 0.76, P < 0.001$); however, the increase in PBF with gestational age is evident even in narrow weight ranges, indicating hyperperfusion of term placentas relative to those earlier in gestation. Fetal weight correlated with measured placental blood flow supplying the corresponding placenta after 50 days gestation ($r = 0.72, P < 0.001$).

Speculation

Placental blood flow appears to be an important independent determinant of spontaneous fetal size. Measurement of uteroplacental blood flow under unstressed conditions accurately describes normal uteroplacental blood flow and may provide more reliable characterizations of the relationships between uteroplacental blood flow, placental size, and fetal growth.

The increase in uterine blood flow during pregnancy and its relationship to the growth of the uterus, placenta and fetus has been studied in a number of relatively large mammals including sheep, goats, and rhesus monkeys (2). These species usually have a small litter size and a relatively small total fetal mass compared to the size of the mother. To our knowledge, there have been no studies of the growth of the uterine blood flow during pregnancy in small mammals studied under chronic steady-state conditions, free of anesthetic or surgical stress. Such data would be of interest from a comparative physiologic point of view because small mammals tend to have large litter sizes and a relatively large total fetal mass in relationship to the size of the mother. The guinea pig is of particular interest in this regard because the normal litter of 4–6 fetuses typically weighs 30–50% of the maternal prepregnant weight (10).

In a previous report it was suggested that the increase in uterine blood flow during pregnancy in the guinea pig is achieved primarily by a redistribution of cardiac output rather than an increase in cardiac output (16); however, little is known about the circulation in the pregnant uterus and placenta and even less about the relationship of placental flow to fetal and placental size. The few studies reported on uteroplacental blood flow in the guinea pig have been carried out under acute experimental conditions (3, 12, 17). Because anesthesia and surgical stress are known to interfere with normal metabolism (9, 19) and cardiovascular function (18, 21), the interpretation of these acute flow data is difficult. The goals of the present study were: (1) to determine uteroplacental blood flow at different stages of gestation in the guinea pig under chronic steady-state conditions; (2) to determine the distribution of uterine flow to placental and nonplacental exchange sites; and (3) to relate placental flow to fetal and placental size.

MATERIALS AND METHODS

Five nonpregnant and 22 pregnant guinea pigs were studied between the 39th and 65th day postconception. The weight of the animals at the time of flow study ranged from 813–965 in nonpregnant animals and from 805–1405 g in pregnant animals. Seven of the pregnant animals were tricolor guinea pigs bred and studied at the Laboratoire de Physiologie du Développement, Collége de France, Paris. All other animals were albino strains obtained from the TNO Institution, Rijkswijk, Holland, and studied according to the same experimental protocol at the University of Nijmegen, Holland.

Animals were anesthetized with ketamine·HCl (60 $mg \cdot kg^{-1}$, I.P.) and xylazine (4 mg I.M.). Catheters (PV1, Bolab Inc., Derry, NH) were inserted into the femoral artery and into the left ventricle (19). The position of the left ventricular catheter was determined at the time of surgery by monitoring the blood pressure waveform, and confirmed at autopsy. The catheters were tunneled subcutaneously and exteriorized between the shoulder blades. Recovery after surgery was verified by determining the daily food intake. Complete restoration of food intake within 3 days was considered as an indication of fetal and maternal well being (19). Catheters were flushed every other day with heparinized saline (250 U/ml). Blood flow measurements were performed between the fifth and sixteenth day after surgery. This time coincided in nonpregnant animals with diestrus and in pregnant animals with a range between the 39th and 65th day postconception.

Uterine and placental blood flow were determined using strontium-85 labeled 15 μ microspheres supplied by 3M Corporation. Organ blood flow and cardiac output were calculated according to the reference sample technique (13). A total of 60,000 microspheres per 100 g maternal weight was injected into the left ventricle via the catheter chronically maintained in the left ventricle. Simultaneously, a reference sample was withdrawn from the abdominal aorta via the femoral arterial catheter. Withdrawal was accomplished using a syringe pump previously calibrated gravimetrically within 0.1%. Typical rates of withdrawal ranged from 0.5–1.0 ml/min. Organ blood flow was calculated by the following formula:

$$\text{Organ flow (ml/min)} = \frac{\text{number of microspheres in organ}}{\text{number of microspheres in reference sample}} \times \text{withdrawal rate} \quad (1)$$

Cardiac output was calculated from the rate of the withdrawal pump and the number of microspheres in the reference sample (13). Cardiac output in this series averaged 266 ± 14 ml/min (270 ± 17 $ml \cdot kg^{-1} \cdot min^{-1}$), in agreement with previous reports (16).

Approximately 30 min after completion of the blood flow measurements, the animals were sacrificed. The catheter position was checked at autopsy and all organs dissected. In all animals, the tip of the left ventricle catheter was located in satisfactory position. In each animal the tip of the abdominal catheter was located between the kidneys and the aortic bifurcation.

In four animals, an adequate reference sample could not be collected. In these animals only the regional distribution of blood flow within the uterus and weights of placentas and fetuses were used in subsequent calculations. The uterus was dissected in the following manner. The placentas were divided into upper and lower portion (11) and placed in preweighed vials in the same order as their location within the uterus. The dissection of the uterine horns, cervix, and vagina was performed as described by Chaichareon and coworkers (5, 16).

Radioactivity in each tissue aliquot and in the reference blood sample was determined with a well scintillation counter adapted with a 3-inch crystal (Packard Autogamma Scintillation Counter 5220). For statistical reasons (4), the actual number of microspheres was calculated in each sample from the cpm/microsphere and the cpm/sample. All uterine, upper placental, and reference samples contained at least 400 microspheres. Good mixing of the microspheres in the present study was supported by a close correlation between the number of microspheres distributed to paired organs (kidney, mammary glands, $r = 0.95$ and $r = 0.93$, respectively).

Statistical significance was judged using the paired or unpaired Student t test as appropriate. Values are expressed as the mean ± S.E. Regression analyses and Chi-square analysis were performed with standard statistical programs.

RESULTS

Distribution of uterine blood flow. The total uterine blood flow expressed per kg of uterus placenta and fetus (minus fluids) did not change consistently with pregnancy in the 18 guinea pigs studied. The mean for all pregnant animals was 171 ± 15 ml$\cdot$kg$^{-1}\cdot$min^{-1} (mean ± S.E.). Placental uterine blood flow represents 93 ± 1% of the total uterine blood flow at term ($n = 8$; gestation ≥ 60 days), and 87 ± 2% of total uterine blood flow in animals less than 60 days gestation. In order to test regional differences in nonplacental uterine blood flow during pregnancy the uterus was analyzed as three separate areas as described by Chaichareon, *et al.* (5). To accommodate differences among animals in absolute flow, the midportion was arbitrarily assigned a value of 100% and the lateral and proximal portions compared to it using the Friedman two-way analysis of variance by Rank (6). It is clear from Figure 1 that the perfusion relationships between areas of the uterus changed from the nonpregnant animals to the mid- and late pregnant animals. In late pregnancy (>56 days) the midportion has significantly lower blood flow than the adjacent lateral and proximal portions of the uterus ($\chi^2 = 6.78$, df = 72, $P < 0.05$). Because of the relatively small number of late pregnant animals with three or more fetuses in one horn, it was not possible to evaluate the role of placental position within the uterus on fetal and placental growth and perfusion.

Placental blood flow versus gestational age. Eighteen animals were studied between 39–65 days gestation (Table 1). Litter size varied among these animals from one to six. Total placental blood flow (Fig. 2a) increases progressively throughout gestation. Animals carrying a litter size of two or less had lower total blood flow than those with larger litter sizes. As demonstrated in Figure 2b, the mean placental blood flow within each litter (ml/min/placenta) remained relatively constant until 50 days gestation. After 50 days gestation, the mean placental blood flow increased with gestation ($r = 0.84$, $P < 0.001$). A similar correlation is seen in Table 1 between gestational age and individual placental blood flows after 50 days ($r = 0.64$, $P < 0.001$).

Placental blood flow versus fetal and placental weights. Figure 3 depicts the relationship between placental blood flow (ml/min) and placental weights for the 59 placentas studied in the 18 pregnant animals. It is clear that large placentas have an increased blood flow ($r = 0.76$, $P < 0.001$). Placental flow per g placental weight (Table 1) remained relatively constant in litters less than 50 days gestation. After 50 days gestation, placental blood flow increased progressively to a term value of approximately 3.0 ml$\cdot$min$^{-1}\cdot$g^{-1}. If one considers the placental blood flow for placentas of the same weight, the variability reflects primarily the impact of gestational age upon placental blood flow. This is illustrated in Figure 4 where placental flow is plotted against gestational age for placentas of a narrow weight range of 4–5 g ($r = 0.80$, $P <$ 0.001). Similar relationships are observed between 3–4 g and

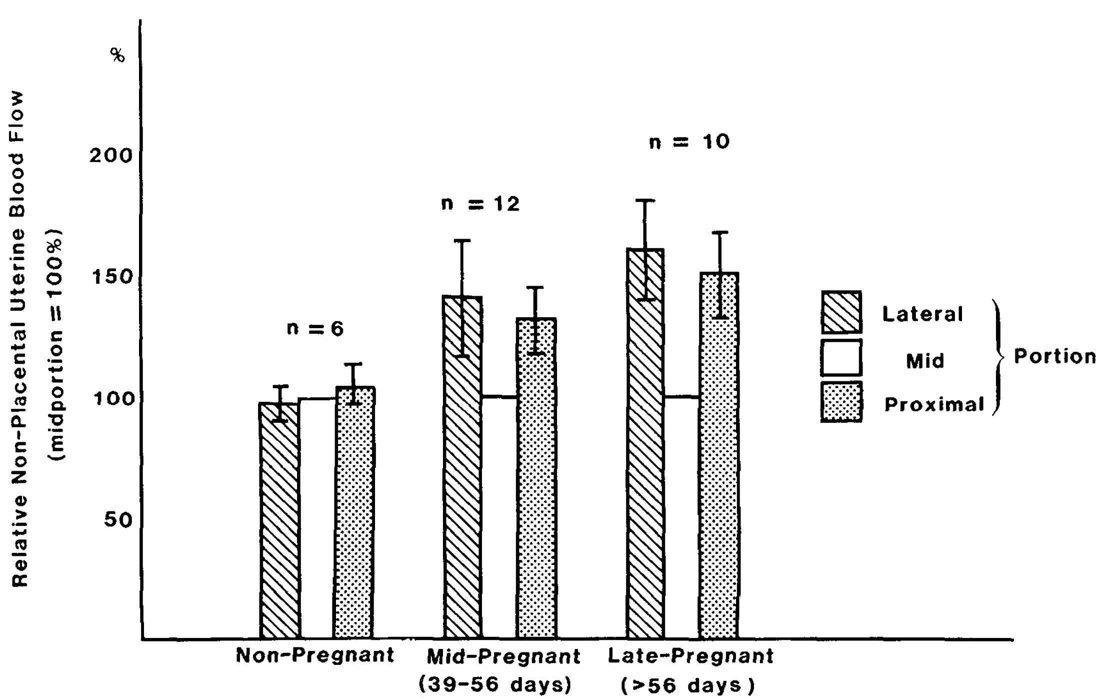

Fig. 1. Blood flow to different regions of the uterus of the nonpregnant and pregnant guinea pig. Values are expressed relative to the flow to the midportion of the uterus (=100%). The vertical bars represent mean ± SE.

Table 1. *Gestational age, fetal and placental weights and placental blood flows for the 18 animals studied*

Getational age (days)	Fetal wt (g)	Placental wt (g)	Placental blood flow ($ml \cdot min^{-1}$)	Placental blood flow ($ml \cdot min^{-1} \cdot g\ plac^{-1}$)	Total uterine blood flow ($ml \cdot kg^{-1} \cdot min^{-1}$)
39	8.9	1.20	0.6	0.50	130
	8.2	1.26	0.4	0.32	
	8.8	0.75	0.4	0.53	
	8.7	1.38	0.9	0.65	
	8.6	1.13	0.6	0.53	
	8.6	1.27	0.7	0.55	
41	10.5	2.28	4.7	2.06	322
	11.5	2.27	4.4	1.94	
	10.5	2.13	4.8	2.25	
	7.8	1.93	3.0	1.55	
43	15.0	3.83	4.8	1.24	243
	15.5	2.59	3.8	1.45	
46	22.7	3.24	6.7	2.07	199
	22.0	3.23	2.9	0.90	
	18.6	2.68	2.5	0.93	
47	22.0	3.63	4.1	1.13	
	21.5	3.37	3.1	0.91	
	21.9	3.16	2.4	0.76	
	23.8	3.81	3.0	0.80	
47	13.0	3.30	6.4	1.94	194
	25.2	2.64	2.5	0.95	
	17.2	2.81	3.1	1.10	
	22.6	2.04	3.1	1.52	
51	40.7	4.41	4.4	1.00	83
	35.7	3.59	2.8	0.78	
	34.3	3.49	2.0	0.58	
	42.5	2.27	4.6	1.09	
	42.8	4.64	3.5	0.74	
53	40.6	5.24	17.4	3.32	263
	37.5	3.81	4.3	1.12	
54	50.8	4.86	8.6	1.76	143
	49.5	3.84	8.2	2.14	
	47.2	3.73	6.7	1.81	
55	60.0	5.22	4.9	0.94	106
	62.3	5.99	8.8	1.47	
56	54.7	5.31	7.5	1.41	129
56	59.0	4.36	6.5	1.49	239
	61.0	4.75	9.1	1.92	
58	59.4	4.72	11.1	2.36	135
	51.0	3.05	5.9	1.93	
	59.3	3.83	7.8	2.03	
	57.0	3.80	8.9	2.35	
	65.8	4.23	9.6	2.27	
60	65.8	5.86	10.3	1.76	137
	62.8	5.14	8.6	1.67	
	60.2	4.93	9.5	1.92	
	62.6	5.81	9.4	1.62	
61	84.0	5.91	12.6	2.14	137
	86.0	6.17	10.7	1.73	
62	73.0	4.47	8.8	2.00	124
	71.1	4.41	8.8	2.00	
	68.4	4.22	7.7	1.82	
63	90.4	6.53	29.7	4.55	205
	85.8	5.79	18.8	3.25	
	72.3	4.97	12.7	2.55	
	77.5	4.66	15.5	3.32	
65	77.1	4.17	12.9	3.10	156
	83.2	4.75	11.3	2.38	
	94.1	5.79	18.2	3.11	

between 5–6 g; thus, among placentas of the same weight, those taken from animals later in gestation have a higher blood flow.

As one might deduce from Figure 2b, there was no apparent relationship between placental blood flow and fetal weight for those gestational ages less than 50 days; however, Figure 5 demonstrates the correlation between placental blood flow and fetal weight obtained after 50 days gestation ($r = 0.72$, $P < 0.001$).

DISCUSSION

The relationships between uterine and placental perfusion on the one hand and fetal and placental growth rates on the other are particularly interesting in the small mammals, which over a relatively short gestation length produce a large total fetal mass and with a large number of fetuses. These characteristics are accen-

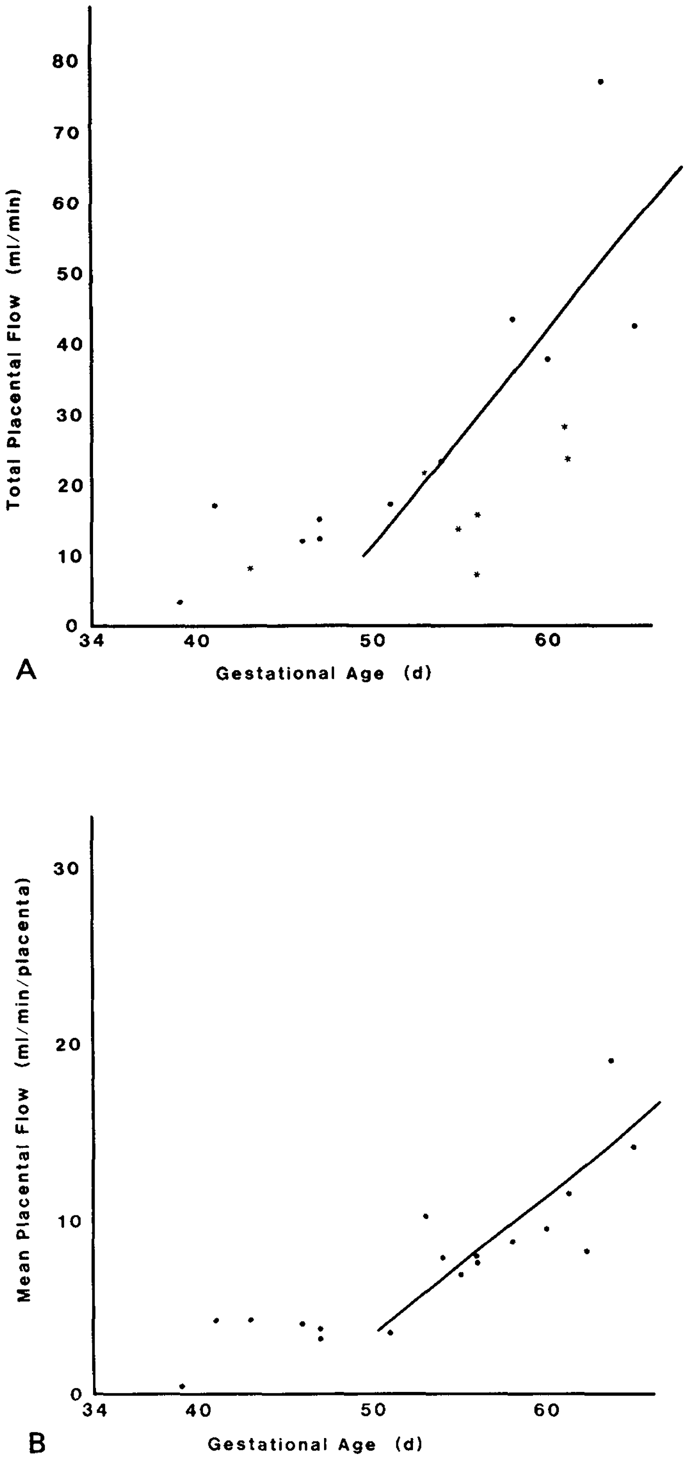

Fig. 2. Placental flow as a function of gestational age in 18 chronically catheterized guinea pigs. *a*), depicts the total placental flow in each litter, with the litters containing one or two fetuses indicated by an "x," and those litters with three or more fetuses indicated by an "o." The relationship between gestational age and total placental blood flow after 50 days gestation in litters of more than two fetuses is shown by the solid line ($r = 0.67$, $P < 0.01$). *b*), depicts the average flow per placenta as a function of gestation. The relationship between gestational age and mean placental blood flow in litters greater than 50 days gestation is shown by the solid line ($r = 0.84$, $P < 0.01$).

tuated in the guinea pig where a typical total fetal mass in a litter with four to six fetuses can equal 30–50% of maternal prepregnant weight (10). Unfortunately, previous studies of uterine blood flow in the guinea pig have been carried out under acute operative and anesthetic stress, conditions which have been shown to alter markedly both maternal metabolism (8, 19) and cardiovascular function (18, 21). Such effects of sampling stress have also been observed in other mammalian species (7, 20).

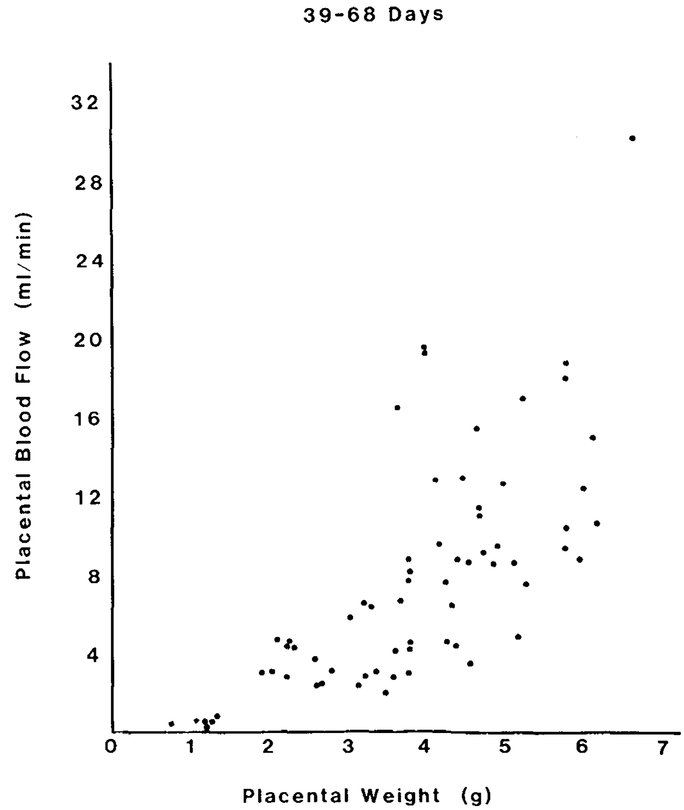

Fig. 3. The relationship of placental blood flow to placental weight in chronically catheterized pregnant guinea pigs.

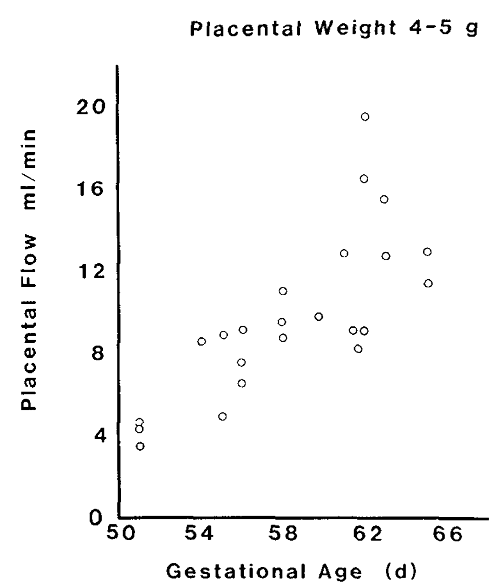

Fig. 4. The relationship of placental blood flow to gestational age in placentas weighing 4–5 g.

The present study describes several features of placental blood flow, which are at variance with previous reports of acutely obtained data. First, the total cardiac output in the present series is approximately 50% higher than reported in acute studies (3, 17). Second, the present series reports a rapid increase in placental blood flow after 50 days gestation, differing qualitatively from acutely obtained data (3, 20). Third, the absolute magnitude of placental blood flow in the present study is almost 100% higher at term than that reported acutely (3). Although methodologic dif-

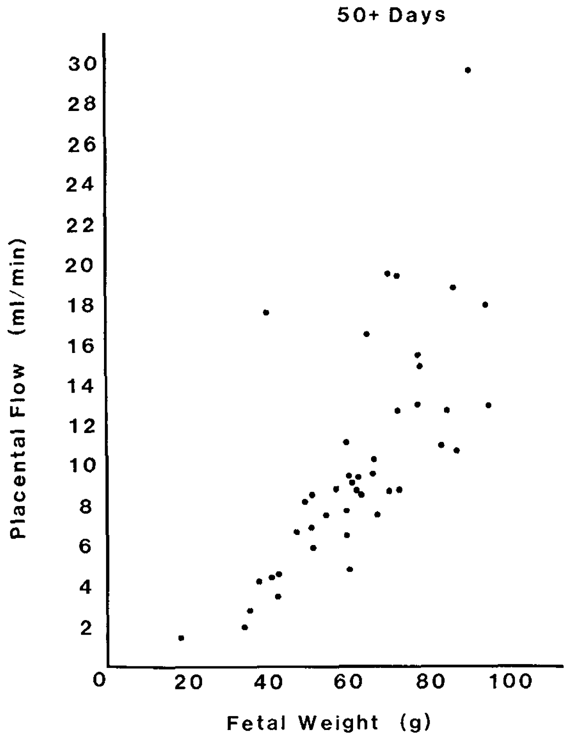

Fig. 5. The relationship between placental blood flow and fetal weight in 36 fetuses from 12 litters of chronically catheterized guinea pigs with gestational age greater than 50 days.

ferences may account for some of the differences within the acute studies, it is evident that both cardiac output and placental blood flow are greatly reduced in the acute studies, relative to the chronic values in the present report. The greater relative reduction in uterine blood flow than cardiac output is consistent with preservation of cardiac output at the expense of maintenance of the pregnancy in response to the unphysiologic stress induced by the acute preparation. Because oxygen, glucose, and amino acids are not strictly flow-limited substrates, the impact of the greater chronic placental blood flow on nutrient fluxes from the mother are not directly predictable (14). It remains also for future investigations to quantitate the impact of the increasing blood flow with gestation on fetal nutrition and growth.

The increase in placental blood flow at 50–55 days is particularly interesting, for two reasons. First, the impact of litter size upon fetal weight becomes evident at this time in gestation. After 50 days gestation, an increasing number of fetuses per litter is associated with decreasing fetal weight (10). Second, maternal glucose concentration falls (19) and both maternal glucose turnover ($mg \cdot min^{-1}$) and glucose clearance ($ml \cdot min^{-1}$) begin to increase substantially after this gestational age, suggesting the impact of increasing fetal mass on maternal metabolism (8); thus, several different physiologic studies point to profound readjustments in maternal physiology at approximately this same stage of gestation.

The total uterine blood flow, expressed per kg of uterine contents less fluids, is relatively constant with gestation and is approximately 171 $ml \cdot kg^{-1} \cdot min^{-1}$. The total uterine flow under unstressed conditions is thus somewhat lower than the values reported in chronically catheterized large mammals. The guinea pig placenta is labyrinthine, with maternal and fetal blood flowing in opposite directions (1, 7, 15). These anatomic findings are supported by the data of Moll and Kastendieck (15), who studied the transfer of nitrous oxide and tritiated water across the placenta, and found an efficient transfer consistent with a countercurrent circulation. It is possible that the countercurrent nature of the flow in the guinea pig permits growth of the total fetal mass at lower absolute flows than in the concurrently-perfused placenta of the large mammal.

Figure 5 demonstrates a linear relationship between placental blood flow and fetal weight from 50 days gestation until term. This increase in blood flow to the placenta after 50 days is apparent even at fixed placental weight ranges, as shown in Figure 4. Thus late gestation placentas are hyperperfused relative to placentas before 50 days gestation, even within a narrow weight range. Prior investigators, using other small mammalian species, have noted that restriction of flow by uterine artery ligation results in fetal growth retardation (22). The present study shows that under unstressed conditions without manipulation of the uterine circulation, the spontaneous fetal weight and placental blood flow are closely related. Further studies are needed to determine the precise relationships between placental flow and fetal weight within litters and to establish any causal relationship between the two.

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24. This research was supported by NIH Program Grant #5-P01-HD00781. J. W. Sparks was supported by NIH Program Training Grant #5-T32-HD07186. F. C. Battaglia was a Josiah Macy Foundation Scholar.
25. Received for publication June 11, 1981.
26. Accepted for publication February 12, 1982.

0031-3998/82/1609-0716$02.00/0