THE EFFECT OF PATERNAL GENOTYPE ON PARITY-DEPENDENT CHANGES IN THE DECIDUAL CELL REACTION, PLACENTAL WEIGHT AND FETAL WEIGHT IN THE MOUSE

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Summary. The extent of the decidual cell reaction on the 7th day of pregnancy in uniparous mice was found to be dependent on the reproductive history of the mother. It is postulated that these observations are due to differences in the mother's immunological status.

Placental weight increased with parity but was unaffected by the sire of the first pregnancy. Fetal weight decreased with parity and there was some suggestion that the mother's immunological status may also affect fetal weight. There was also an apparent association between a reduction in decidual weight on the 7th day and a reduction of fetal weight on the 18th day of pregnancy.

INTRODUCTION

Placental weight and birth weight increase with parity in man (Calkins, 1937; Karn & Penrose, 1951; Juyant, 1966) and in other mammals (Broadhurst & Jinks, 1965; McCarthy, 1965). Parity also affects a mother's immunological status. In humans, a mother may become sensitized to fetal erythrocytes (Freda, 1965) and leucocytes (Jensen, 1962; Brain, 1968). Parous female mice may possess both humoral- and cell-bound immunity to the male strain antigens (Goodlin & Herzenberg, 1964; Kaliss & Dagg, 1964; Soren, 1967) but, in contrast, may exhibit unresponsiveness with respect to paternal antigens (Breyere & Barrett, 1960; Kaliss & Dagg, 1964; Hellström, Hellström & Brawn, 1969).

James (1965) has postulated that, when a female mouse is tolerant to paternal antigens, placental and fetal weight are less than in controls, while, if females are made sensitive to paternal antigens, placental and fetal weight is increased. If this is true, then the effects of parity might have an immunological basis. Clarke (1971), using mice of the same strains, has been unable to confirm the work of James, and Boshier & Moriarty (1970) found that pre-immunization of a ewe to the antigens of the mating ram did not affect placental weight in sheep. Recent work on the mouse has shown that decidual weight on the 7th day of pregnancy is reduced in females sensitized to paternal antigens (Clarke & Hetherington, 1971).

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If the effects of parity are due to the mother's immunological status with respect to paternal antigens, then altering the sire between pregnancies should modify parity dependent changes in placental and fetal weight. If sensitization to paternal antigens reduces decidual weight on the 7th day of pregnancy, parity may also affect this measurement.

Decidual weight on the 7th day, and placental and fetal weight on the 18th day were studied, with a view to determining whether immunological factors were associated with the changes in these measurements associated with parity.

MATERIALS AND METHOD

Mice of JU/Fa, C57BL/McL and CBA/Fa strains were used. Inbred mice are referred to as JU, C57 or CBA. The conceptuses derived from mating a JU female to a CBA male are designated as JU.CBA and those from the reciprocal cross as CBA.JU. Uniparous JU mice are referred to as JU (CBA) where the strain in brackets refers to the strain of the sire of the first pregnancy.

Mice were killed on either the 7th or 18th day of their first or second pregnancy. The day of finding the vaginal plug was taken as the first day of pregnancy. All virgin mice were mated at 8 weeks of age. Those which were used to provide data on second pregnancies were allowed to rear their litters, and were remated at about 14 weeks of age.

The mating system used for JU mice was as follows:

<i>Sire of first pregnancy</i>	<i>Sire of second pregnancy</i>
JU	JU
JU	CBA
JU	C57
C57	JU
C57	C57
CBA	JU
CBA	CBA

A similar scheme was used for the other two strains.

Decidual weights on the 7th day of pregnancy and placental and fetal weights on the 18th day were obtained as described previously (Hetherington, 1971). Analysis of placental and fetal weight was by regression on litter size. Decidual weights were found to be unaffected by litter size and were, therefore, compared by analyses of variance.

RESULTS

Parity affected decidual weight in all three strains. In the majority of instances, decidual tissue in uniparous mice was significantly heavier than in nulliparous mice (Table 1). Within JU uniparous females, however, the mean decidual weight induced by JU.CBA blastocysts in JU(CBA) mothers was significantly greater than that induced in JU(JU) mothers ($P = 0.05$). The difference in mean decidual weight induced by JU.JU blastocysts in JU and JU(JU) mothers was also significantly less than the difference in decidual weight induced by

JU.CBA blastocysts, in JU and JU(CBA) mothers ($P = 0.05$). It appears, therefore, that the increase in decidual weight associated with JU.CBA blastocysts is not only the non-specific effect of parity, or age, but is also due to the genotype of the litter of the first pregnancy.

As in JU mice, mean decidual weight in uniparous C57 mice was greater than in nulliparous C57 mice, this effect being significant in the majority of instances. The mean decidual weight associated with C57.JU blastocysts was greater in C57 (JU) than in C57(C57) mothers ($P = 0.05$).

TABLE 1
DECIDUAL WEIGHTS IN NULLIPAROUS AND UNIPAROUS JU, C57 AND CBA MICE

Dam	Nulliparous			Sire of first pregnancy	Uniparous		
	Sire				Sire		
	JU	C57	CBA		JU	C57	CBA
JU	5.23 ± 0.17 (33)	5.02 ± 0.14 (34)	5.87 ± 0.11 (30)	JU	5.62 ± 0.11 (26)	5.80 ± 0.17 (24)	6.38 ± 0.22 (25)
				C57	5.84 ± 0.18 (20)	6.14 ± 0.20 (20)	
				CBA	5.54 ± 0.26 (15)		7.52 ± 0.20 (15)
C57	4.87 ± 0.20 (22)	4.66 ± 0.21 (24)	5.06 ± 0.20 (25)	JU	6.07 ± 0.15 (15)	5.12 ± 0.17 (15)	
				C57	5.34 ± 0.17 (24)	5.59 ± 0.21 (24)	5.90 ± 0.14 (25)
				CBA		5.38 ± 0.19 (14)	5.84 ± 0.32 (14)
CBA	4.14 ± 0.10 (15)	5.28 ± 0.17 (15)	4.20 ± 0.18 (20)	JU	5.15 ± 0.20 (12)		4.09 ± 0.23 (12)
				C57		4.26 ± 0.13 (12)	4.19 ± 0.20 (12)
				CBA	4.66 ± 0.14 (14)	5.05 ± 0.18 (15)	5.06 ± 0.21 (16)

Results expressed as mean decidual weight in mg $\pm$ 1 S.E.; group size in parentheses.

The situation in CBA mice was more complex. In certain instances, an increase in mean decidual weight occurred between nulliparous and uniparous mice, e.g. between CBA.CBA and CBA (CBA) CBA blastocysts ($P = 0.01$) whilst, in other instances, mean decidual weight decreased, e.g. between CBA.C57 and CBA (C57) C57 blastocysts ($P = 0.05$). As no decrease was apparent in decidual weight between CBA.C57 and CBA (CBA) C57, this decrease is apparently dependent on the sire of the first pregnancy. The CBA.CBA blasto-

cysts also induced a smaller response in both CBA(JU) and CBA (C57) mothers than in CBA (CBA) mothers ($P = 0.05$). A similar reduction in decidual weight associated with an inbred blastocyst following a hybrid pregnancy is apparent in C57 mothers and in JU (CBA) mothers but in these instances the effect was not significant. The mean decidual weight induced by CBA (JU) JU blastocysts was also greater than that induced by CBA (CBA) JU blastocysts, and although, in this instance, the difference was not significant, the situation appears to be analogous to that associated with JU (CBA) and C57 (JU) mothers.

TABLE 2

PLACENTAL WEIGHT IN NULLIPAROUS AND UNIPAROUS JU, C57 AND CBA MICE

Dam	Nulliparous			Sire of first pregnancy	Uniparous		
	Sire				Sire		
	JU	C57	CBA		JU	C57	CBA
JU	148.51 ± 4.5 (8.0±0.6)	144.5 ± 2.3 (8.0±0.5)	130.2 ± 1.9 (8.7±0.3)	JU	153.9±3.2 (9.1±0.5)	152.1±2.0 (10.4±0.5)	140.8±3.1 (10.5±0.5)
				C57	143.8±2.6 (10.8±0.4)	148.0±3.7 (9.7±0.6)	
				CBA	157.3±3.5 (8.8±0.7)		141.1±3.5 (10.0±0.5)
C57	131.6±3.4 (6.6±0.3)	98.1±2.2 (6.8±0.4)	106.9±1.8 (6.9±0.5)	JU	133.2±2.8 (7.5±0.4)	102.2±2.8 (6.8±0.7)	
				C57	135.5±2.7 (8.2±0.6)	98.9±2.7 (8.2±0.5)	111.7±4.0 (7.7±0.6)
				CBA		95.7±2.4 (8.0±0.4)	113.0±1.9 (8.7±0.7)
CBA	139.9±4.3 (5.9±0.6)	124.7±2.2 (6.8±0.5)	104.7±1.7 (6.8±0.5)	JU	140.9±3.6 (7.4±0.5)		111.1±3.1 (8.0±0.4)
				C57		122.2±2.0 (8.1±0.4)	111.6±3.3 (6.9±0.4)
				CBA	139.1±3.0 (7.0±0.7)	118.4±2.3 (7.5±0.4)	111.4±2.5 (7.1±0.7)

Results expressed as mean placental weight in mg (uncorrected for litter size) ± 1 S.E.; mean litter size ± 1 S.E. in parentheses.

Placental weights in uniparous mice were generally heavier than placentae of the same genotype in nulliparous mice (Table 2). The sire of the first pregnancy had no significant effect on placental weight in the subsequent pregnancy. By contrast, fetal weight (Table 3) was generally less in uniparous mice than in identical matings involving nulliparous mice, and was affected by the sire of the first pregnancy.

In every instance except JU (C57) JU, the mean fetal weight of inbred litters following a hybrid pregnancy after correction for litter size, was less than the fetal weight of inbred litters following an inbred pregnancy, the difference being significant in C57 (JU) and C57(CBA) mothers ($P = 0.05$ and 0.05). The mean fetal weight of hybrid fetuses following either an inbred or hybrid pregnancy

did not differ consistently in any way, nor were any significant differences found.

TABLE 3
FETAL WEIGHT IN NULLIPAROUS AND UNIPAROUS JU, C57 AND CBA MICE

Dam	Nulliparous			Sire of first pregnancy	Uniparous		
	Sire				Sire		
	JU	C57	CBA		JU	C57	CBA
JU	727.9±12.1 (8.0±0.6)	800.3±13.9 (8.0±0.5)	818.3±12.4 (8.7±0.3)	JU	703.6±11.2 (9.1±0.5)	828.5±11.3 (10.4±0.5)	830.9±10.3 (10.5±0.5)
				C57	701.4±7.7 (10.8±0.4)	804.3±16.14 (9.7±0.6)	
				CBA	679.9±9.42 (8.8±0.7)		846.49±13.55 (10.0±0.5)
C57	863.36±16.5 (6.6±0.3)	759.5±19.3 (6.8±0.4)	804.4±29.4 (6.9±0.5)	JU	781.2±32.2 (7.5±0.4)	657.4±26.7 (6.8±0.7)	
				C57	795.7±15.7 (8.8±0.6)	740.4±17.4 (8.2±0.5)	794.3±17.6 (7.7±0.6)
				CBA		641.5±22.5 (8.0±0.4)	798.5±14.9 (8.7±0.7)
CBA	763.9±20.4 (5.9±0.6)	747.8±21.1 (6.8±0.5)	667.5±18.5 (6.8±0.5)	JU	692.5±15.5 (7.4±0.5)		595.4±11.7 (8.0±0.4)
				C57		704.2±9.9 (8.1±0.4)	610.1±14.3 (6.9±0.4)
				CBA	719.8±24.9 (7.0±0.7)	727.4±13.1 (7.5±0.4)	619.2±17.9 (7.1±0.7)

Results expressed as mean fetal weight in mg (uncorrected for litter size) ± 1 S.E.; mean litter size ± 1 S.E. in parentheses.

DISCUSSION

In general, decidual weights increased from first to second pregnancy. The difference in maternal weight and age may be partly responsible. The greater response to JU.CBA blastocysts in JU (CBA) than in JU (JU) mothers and the similar increase in response associated with C57.JU blastocysts in C57(JU) females when compared with C57(C57) females (Table 1), indicates that the decidual cell reaction in uniparous mice is affected by the sire of the first pregnancy. An effect of the sire of the first pregnancy may also be seen in the decrease in the decidual response associated with CBA.C57 blastocysts in CBA (C57) mothers when compared with CBA (CBA) mothers.

Maternal pre-immunization to paternal antigens reduced decidual weight on the 7th day of pregnancy (Clarke & Hetherington, 1971). The reduction in decidual weight associated with CBA.C57 blastocysts may indicate sensitization to C57 antigens after a pregnancy by a C57 male. The increase in decidual weight associated with C57.JU and JU.CBA blastocysts may be the result of maternal unresponsiveness to paternal antigens.

Placental and fetal weight are positively correlated with maternal weight (McCarthy, 1965). The increase in placental weight observed between nulli-

parous and uniparous mice (Table 2) may therefore be partly the result of differences in maternal weight. Fetal weight, however, tended to be lighter in uniparous mice (Table 3) and confirms the data of McCarthy (1965). The absence of an effect of the sire of the first pregnancy on placental weight despite the apparent changes in the maternal immunological status as detected by the decidual cell reaction, is contrary to the observations of James and confirms the observations of Clarke (1971) that placental weight is unaffected by prior presensitization of the mother to the paternal antigens. This also suggests that the increase in placental weight associated with parity is not related to changes in the mother's immunological status.

The absence of a direct effect of antigenic differences on fetal weight is perhaps not surprising, as placental weight was similarly unaffected. The reduction in fetal weight in an inbred pregnancy following a hybrid pregnancy, compared with that in an inbred pregnancy following an inbred pregnancy and the observation that decidual weight was reduced in the same circumstances indicates that these two parameters may be correlated. A similar association between decidual and fetal weight in the absence of an effect on placental weight was found by Hetherington (1971) and is apparent in comparisons between the work of Clarke (1971) and Clarke & Hetherington (1971).

It is not known whether the reduction in fetal weight is the direct effect of changes in the decidual cell reaction, or whether both are dependent on a third factor. The reason for the reduction in decidual weight in the above situation must be speculative. Changes in the mother's immunological status with respect to paternal antigens of the first pregnancy are not possible where the first pregnancy was hybrid and the second, inbred. Synergism between 'weak' and 'strong' antigens, however, occurs in chick red blood cells (Shierman & McBride, 1967) and in hamster/Ehrlich ascites tumour hybrid cell lines (Watkins & Chen, 1969), and Kirby (1968) reported that, after the extrauterine development of allogeneic mouse blastocysts, the development of syngeneic blastocysts was inhibited. It is possible, therefore, that the reduction in decidual weight recorded here may indicate that a synergism between 'strong' antigens and 'weak' tissue-specific antigens may occur during pregnancy, and may affect subsequent decidual and fetal development.

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