J. Reprod. Fert. (1971) **25**, 417–424

THE DECIDUAL CELL REACTION, PLACENTAL WEIGHT, FOETAL WEIGHT AND PLACENTAL MORPHOLOGY IN THE MOUSE

C. M. HETHERINGTON*

Institute of Animal Genetics, Edinburgh

(*Received* 22*nd July* 1970)

Summary. The extent of the decidual cell reaction (DCR) on the 7th day of pregnancy and placental weight, foetal weight and placental morphology on the 18th day were found to be dependent on the genotype of the conceptus and the environment provided by the mother. No association could be found between the extent of the DCR and placental weight or placental morphology. There was, however, some suggestion of a positive association between decidual weight and foetal weight.

It is suggested that, in the presence of antigenic differences between mother and conceptus, the ability of a conceptus to elicit the DCR is impaired, or the rate of development of the decidual tissue is reduced.

INTRODUCTION

The decidual cell reaction (DCR) in the uterine stroma is one of the characteristic features of ovum implantation in the mouse. This cellular proliferation occurs in the proximity of a blastocyst, and may be induced artificially. It is therefore possible, by the injection of arachis oil (Finn & Hinchliffe, 1964), to measure the ability of a uterus, of a given genotype, to respond to a decidual stimulus in the absence of a blastocyst.

The function of the DCR is uncertain. It may act as a source of nutrition for the developing conceptus, contain the invasiveness of the trophoblast (Kirby, 1965), or protect the conceptus from immunological rejection by the mother during the early days of pregnancy (Kirby, Billington & James, 1966). It was of interest, therefore, to study the effect of conceptus and maternal genotype on the extent of the DCR and to determine whether the degree of decidual cell formation in early pregnancy was related to foetal weight, placental weight and placental morphology in later pregnancy.

MATERIALS AND METHODS

Mice

Inbred JU/Fa, C57BL/McL and CBA/Fa mice of the Institute of Animal Genetics, Edinburgh, and F_1 mice derived from them were used. All females

* Present address: Clinical Research Centre, Northwick Park, Harrow, Middlesex.

were 8 weeks old at the time of mating. Pseudopregnancy was induced by mating females to vasectomized males. The day of finding the vaginal plug was taken as the 1st day of pregnancy or pseudopregnancy.

Nomenclature

Inbred mice are referred to as JU, C57 or CBA. The conceptuses and F_1 mice derived from mating a JU female to a CBA male are designated as (JU.CBA) while those from the reciprocal cross are designated as (CBA.JU). Crosses involving mice of other genotypes are described in a similar fashion.

Deciduoma induction

Deciduomata were induced by the injection of 0·01 ml of arachis oil into the lumen of each uterine horn on the 4th day of pseudopregnancy at 13.00 hours. Mice were killed on the morning of the 7th day of pseudopregnancy. Uteri were trimmed of their mesenteries, blotted lightly and weighed to the nearest milligram.

The decidual cell reaction

Inbred and F_1 females were mated to JU, C57 or CBA males. The decidual tissue present on the 7th day of pregnancy was dissected free from the uterus, under physiological saline, blotted lightly and weighed to the nearest milligram. No attempt was made to remove the tissue of the conceptus. The mean weight of decidual tissue per implantation site was calculated.

Rate of conceptus development

Mice were killed at 15.00 hours on the 4th day of pregnancy. Their uteri were flushed, and the numbers of morulae and blastocysts were recorded.

Foetal weight, placental weight and placental morphology

Placentae and foetuses were dissected free from their membranes on the 18th day of pregnancy, blotted lightly and weighed to the nearest milligram. The mean foetal and placental weights per female were calculated and used in the analysis.

Four placentae from each of five litters were selected at random from each cross. The placentae were fixed in Bouin's fixative. Sections, 8 μm thick, were stained with haematoxylin and eosin. Midline placental sections were drawn with the aid of a camera lucida, and the area of trophoblast and decidual tissue was measured with a planimeter.

RESULTS

The uterine response of JU mice to the intraluminal injection of arachis oil was significantly greater than the response of either C57 or CBA mice (Table 1, $P<0·05$). There was no significant difference between the responses of JU.C57, JU.CBA and C57.CBA females. No significant difference was found between genotypes when uterine weight on the 7th day was expressed as a proportion of uterine weight on the 4th day.

Significant differences were found in the mean weight of decidual tissue associated with conceptuses of different genotypes within the same maternal environment and with conceptuses of the same genotype in different environments (Table 2). Within JU females, the mean decidual weight induced by JU.CBA conceptuses was significantly heavier than that induced by either JU.JU or JU.C57 conceptuses ($P<0{\cdot}05$) and was also significantly heavier than

TABLE 1

MEAN UTERINE WEIGHT ON THE 4TH AND 7TH DAYS OF PSEUDOPREGNANCY AFTER DECIDUOMATA INDUCTION BY OIL

Genotype of female	*Mean uterine wt (mg ± S.E.) (Five mice/group)*		*Mean % increase in uterine wt between the 4th and 7th day*
	4th day	*7th day*	
JU	94·8 ± 3·3	604·4 ± 78·3	586·5 ± 37·1
C57	63·2 ± 3·2	410·0 ± 51·8	648·7 ± 82·0
CBA	69·7 ± 4·1	417·5 ± 40·8	599·3 ± 58·6
JU.CBA	86·7 ± 2·7	569·0 ± 62·6	656·2 ± 72·2
JU.C57	80·0 ± 1·8	556·8 ± 35·2	696·0 ± 44·0
C57.CBA	76·8 ± 1·4	566·3 ± 45·8	737·9 ± 59·6

TABLE 2

DECIDUAL WEIGHT IN INBRED AND F_1 HYBRID FEMALES

Genotype of dam	*Genotype of sire*					
	JU		*C57*		*CBA*	
	No. of mice	*Mean decidual wt (mg ± S.E.)*	*No. of mice*	*Mean decidual wt (mg ± S.E.)*	*No. of mice*	*Mean decidual wt (mg ± S.E.)*
JU	33	5·23 ± 0·17	34	5·02 ± 0·14	30	5·87 ± 0·11
C57	22	4·87 ± 0·20	24	4·66 ± 0·21	25	5·06 ± 0·20
CBA	15	4·14 ± 0·10	15	5·28 ± 0·17	20	4·20 ± 0·20
JU.CBA	15	5·65 ± 0·13	13	4·79 ± 0·13	15	6·83 ± 0·18
CBA.JU	12	5·12 ± 0·21	8	4·46 ± 0·27	9	5·59 ± 0·25
JU.C57	20	7·18 ± 0·29	20	6·28 ± 0·12	12	5·91 ± 0·26
C57.JU	12	7·05 ± 0·18	12	6·48 ± 0·22	13	6·05 ± 0·27
C57.CBA	14	5·68 ± 0·14	15	5·96 ± 0·19	16	6·43 ± 0·11
CBA.C57	12	5·38 ± 0·24	12	5·97 ± 0·20	12	5·92 ± 0·16

the response induced by a conceptus of the same genotype in CBA females ($P<0{\cdot}01$). In CBA females, the response to CBA.C57 conceptuses was greater than that of either CBA.CBA or CBA.JU conceptuses ($P<0{\cdot}05$). No significant difference within C57 females was apparent. Within F_1 females of a given genotype, the differences between the decidual response to conceptuses of different genotypes were significant in all instances. When an F_1 female was mated to an unrelated strain, the mean decidual weight was always less than when the female was mated to a parental strain. Thus, mean decidual weight

in JU.CBA and CBA.JU females, after mating with a C57 male, was significantly lighter than after mating with either CBA or JU males ($P<0{\cdot}01$). Similarly, the mean decidual weight of (JU.C57)CBA, (C57.JU)CBA, (C57.CBA)JU and (CBA.C57)JU conceptuses was the lightest in each within-mother comparison ($P<0{\cdot}05$ and $P<0{\cdot}05$, respectively). An effect of maternal environment not directly attributable to genotype was also apparent in F_1 females, this being most marked in comparisons between JU.CBA and CBA.JU females where mean decidual weights in CBA.JU females were significantly lighter than in JU.CBA females ($P<0{\cdot}05$).

No significant difference in the rates of development of conceptuses of different genotypes and no consistent association between rate of development and decidual weight was found in within-mother comparisons (Table 3).

As with decidual weight, foetal and placental weights were dependent on the genotype of the conceptus and the maternal environment (Table 4). Crossbred foetuses of inbred mothers were heavier than inbred foetuses, and a similar

TABLE 3

DEVELOPMENT STAGE OF THE CONCEPTUS AT THE TIME OF MAXIMUM UTERINE SENSITIVITY FOR THE INDUCTION OF THE DECIDUAL CELL REACTION

Genotype of female	*Genotype of male*	*No. of mice*	*No. of morulae and blastocysts*	*% blastocysts*	*Decidual wt (mg ± S.E.)*
JU	JU	3	24	83·3	5·23 ± 0·17
JU	CBA	3	32	65·6	5·87 ± 0·11
JU.CBA	JU	8	48	41·6	5·63 ± 0·13
JU.CBA	C57	5	25	28·0	4·79 ± 0·13
JU.CBA	CBA	9	77	38·9	6·83 ± 0·18
C57.JU	JU	7	40	85·0	8·05 ± 0·18
C57.JU	C57	5	33	87·9	6·48 ± 0·22
C57.JU	CBA	3	38	86·8	6·65 ± 0·27

situation with respect to placental weight was found in C57 and CBA females. In JU females, however, JU.C57 placentae did not differ significantly in weight from JU.JU placentae while JU.CBA placentae, after correction for litter size, were significantly lighter than inbred placentae ($P<0{\cdot}05$).

In comparisons within mothers, there was no consistent association between decidual weight on the 7th day of pregnancy (Table 2) and either placental weight (Table 4) or placental morphology (Table 5) on the 18th day. For example, CBA.C57 placentae had a greater decidual and placental weight than CBA.CBA conceptuses, but a smaller decidual area, while JU.CBA conceptuses induced the largest decidual response in JU mothers, but had the lightest placentae with the smallest decidual area. This lack of association between decidual weight and area is apparent in other situations and in comparisons between decidual weight and trophoblast area.

While decidual weight on the 7th day of pregnancy was not associated with placental measurements on the 18th day, there was an association between

TABLE 4

FOETAL AND PLACENTAL WEIGHTS AND NUMBER OF LIVE IMPLANTS IN INBRED AND F_1 HYBRID FEMALES

Genotype of dam	*Genotype of sire*											
	JU				*C57*				*CBA*			
	No. of mice	*Implant no.* (±*S.E.*)	*Placental wt* (*mg*±*S.E.*)	*Foetal wt* (*mg*±*S.E.*)	*No. of mice*	*Implant no.* (±*S.E.*)	*Placental wt* (*mg*±*S.E.*)	*Foetal wt* (*mg*±*S.E.*)	*No. of mice*	*Implant no.* (±*S.E.*)	*Placental wt* (*mg*±*S.E.*)	*Foetal wt* (*mg*±*S.E.*)
JU	35	8·0±0·6	148·5±0·45	727·9±12·1	36	8·0±0·5	144·5±2·3	800·3±13·9	35	8·7±0·3	130·2±1·9	818·3±12·4
C57	21	6·6±0·3	131·6±3·4	863·4±16·5	22	6·8±0·4	98·1±2·2	759·9±19·3	20	6·9±0·5	106·9±1·8	804·4±29·4
CBA	12	5·9±0·6	139·9±4·3	763·9±20·4	13	6·8±0·5	124·7±2·2	747·8±21·1	12	6·8±0·5	104·7±1·7	667·5±18·5
JU.CBA	25	7·0±0·2	126·6±3·0	780·8±15·1	26	8·7±0·4	112·2±2·1	802·9±3·6	12	7·7±0·5	108·0±1·2	845·0±11·3
CBA.JU	10	9·0±0·6	119·5±2·9	777·0±32·6	11	7·5±0·6	115·2±3·0	762·0±25·4	9	6·7±0·8	115·6±3·3	760·2±23·6
JU.C57	31	8·7±0·2	118·7±1·4	900·0±10·2	28	8·0±0·4	107·1±1·6	936·4±9·2	12	8·2±0·5	115·8±1·7	903·0±13·3
C57.JU	16	8·5±0·4	116·1±2·1	848·3±12·5	15	7·9±0·5	101·3±1·9	891·0±14·0	12	8·8±0·4	107·5±2·0	921·8±16·2
C57.CBA	15	7·4±0·4	116·1±2·0	898·6±25·1	17	7·0±0·2	92·0±1·5	825·1±17·3	19	6·8±0·4	98·4±2·4	885·3±10·7
CBA.C57	12	7·5±0·4	118·2±2·4	891·3±10·9	12	7·1±0·6	92·2±1·7	796·7±11·4	12	8·0±0·6	94·9±2·6	795·7±26·7

decidual and foetal weight. The maternal environment of CBA.JU mothers, relative to that of JU.CBA mothers, reduced decidual weight on the 7th day of pregnancy and restricted foetal growth, as measured by foetal weight on the 18th day, but had no effect on placental weight. Similarly, in comparisons between CBA.C57 and C57.CBA mothers, no significant differences in placental weight were apparent although (C57.CBA)CBA decidual and foetal weights were significantly heavier than those of (CBA.C57)CBA conceptuses ($P<0.01$). No significant differences in decidual weight between JU.C57 and C57.JU mothers were found, however, although differences in foetal weight occurred, but in this instance placental weight also differed. Directly analagous comparisons in inbred mothers are not possible due to genetic differences between mothers in reciprocal crosses, but in the comparison between JU.CBA and

TABLE 5

AREAS OF TROPHOBLAST AND DECIDUAL TISSUE FROM MIDLINE PLACENTAL SECTIONS

Genotype of dam	*Genotype of sire*					
	JU		*C57*		*CBA*	
	Trophoblast area ($mm^2 \pm S.E.$)	*Decidual area* ($mm^2 \pm S.E.$)	*Trophoblast area* ($mm^2 \pm S.E.$)	*Decidual area* ($mm^2 \pm S.E.$)	*Trophoblast area* ($mm^2 \pm S.E.$)	*Decidual area* ($mm^2 \pm S.E.$)
JU	10·82 ± 0·27	2·31 ± 0·19	10·42 ± 0·32	2·22 ± 0·16	10·52 ± 0·35	2·06 ± 0·06
C57	9·84 ± 0·71	2·16 ± 0·72	7·32 ± 0·40	1·79 ± 0·07	8·77 ± 0·33	1·90 ± 0·14
CBA	10·38 ± 0·09	2·26 ± 0·15	9·66 ± 0·37	2·00 ± 0·15	9·83 ± 0·44	1·75 ± 0·16
JU.CBA	10·59 ± 0·55	2·46 ± 0·15	8·47 ± 0·40	1·80 ± 0·05	9·53 ± 0·36	1·92 ± 0·06
CBA.JU	8·37 ± 0·18	1·92 ± 0·05	8·68 ± 0·39	2·03 ± 0·13	8·46 ± 0·32	1·72 ± 0·12
JU.C57	9·94 ± 0·42	2·07 ± 0·12	9·19 ± 0·33	1·87 ± 0·08	9·60 ± 0·28	2·27 ± 0·07
C57.JU	8·49 ± 0·41	1·99 ± 0·11	7·98 ± 0·51	1·96 ± 0·11	7·89 ± 0·20	1·87 ± 0·09
C57.CBA	8·95 ± 0·29	2·17 ± 0·11	7·01 ± 0·05	1·84 ± 0·10	7·77 ± 0·14	1·58 ± 0·07
CBA.C57	9·12 ± 0·25	2·00 ± 0·08	7·70 ± 0·61	1·80 ± 0·06	8·02 ± 0·27	1·84 ± 0·09

CBA.JU conceptuses, where there was no difference in placental weight, both decidual and foetal weights were heavier in JU mothers. In the other reciprocal crosses, the differences in decidual weight were not significant, and, although foetal weights differed, so too did placental weights.

DISCUSSION

The extent of the DCR will depend not only on the period of time from induction to autopsy, but also on the strength of the induction stimulus and the ability of the uterus to respond.

Significant differences between genotypes in uterine responsiveness to DCR induction by oil were apparent, as too were variations in the maternal environment not directly dependent on genotype. The rate of increase in uterine weight after DCR induction by oil, however, did not differ significantly between females of different genotypes.

The time of uterine sensitivity to DCR induction is maternally controlled and probably independent of the genotype of the conceptus. Differences in

decidual weight, therefore, within mothers of a given genotype, indicate that either the strength of the decidual stimulus, or the rate of decidual development, is affected by the genotype of the conceptus. The stage of development of the conceptus at the time of uterine sensitivity to DCR induction may affect the strength of stimulus, but in three instances, where differences in decidual weight were detected, there was no consistent association, within mothers of a given genotype, between decidual weight and the stage of development of the conceptus (Table 3). Thus, although in certain strain combinations the rate of development of the conceptus may be affected by its genotype (McLaren, 1968), differences in decidual weight not associated with this factor occur, and a conceptus may therefore exhibit heterosis in its ability to produce the DCR.

The data obtained from F_1 mothers are of particular interest for, in every instance, the decidual weight was greater when the sire was of a parental strain than when the sire was of an unrelated strain. The decidual stimulus or the subsequent rate of DCR development was, therefore, less when the conceptus was derived from a mating with an unrelated strain than when the conceptus was derived from a backcross.

Conceptuses derived from mating with an unrelated strain are outbred while those from a backcross are, on average, 50% inbred. The 'vigour' of the outbred conceptuses might be expected to be greater than that of 50% inbred conceptuses although evidence from other sources suggests that this difference may be small (Robertson & Reeve, 1955; Sved, Reed & Bodmer, 1967). The decidual response induced by the outbred conceptus, however, was the smallest in all instances.

Outbred conceptuses differ antigenically from their mothers while conceptuses derived from a backcross possess no antigens that are not present in the mother. It is possible, therefore, that when mother and conceptus differ antigenically, the DCR is reduced. In order, therefore, that hybrid vigour in the decidual response may be detected, the increase in response due to hybrid vigour must be greater than the reduction due to the antigenic differences between mother and conceptus.

It is postulated that the reduction in decidual response, in the presence of antigenic differences between mother and conceptus, reflects an immunological interaction which impairs the ability of the conceptus to elicit the DCR or reduces the rate of development of the DCR.

It has been postulated (Billington, 1964) that hybrid placentae are heavier due to antigenic differences between mother and conceptus, and that heterosis, as such, has little to do with the increase in placental weight associated with cross-breeding (McLaren, 1965; McCarthy, 1965). In JU mothers, however, JU.C57 placentae did not differ in weight from JU.JU placentae and JU.CBA placentae were significantly lighter. While in F_1 mothers, where differences attributable to heterosis are reduced, placental weight in the presence of antigenic differences was heaviest only in C57.CBA mothers.

There was no apparent association between decidual weight on the 7th day of pregnancy and either placental weight or placental morphology. However, the positive association between decidual weight and foetal weight in all comparisons where placental weight remained constant lends support to the

concept that the DCR may act as a source of nutrition during early pregnancy. It is possible, however, that foetal weight is not greater as a result of the more extensive DCR, but that both decidual and foetal weight are dependent on a third factor.

ACKNOWLEDGMENTS

I am grateful to Dr Anne McLaren for advice, to the Ford Foundation for equipment and to the Medical Research Council for financial support.

REFERENCES

BILLINGTON, W. D. (1964) Influence of immunological dissimilarity of mother and foetus on size of placenta in mice. *Nature, Lond.* **202,** 317.

FINN, C. A. & HINCHLIFFE, J. R. (1964) Reaction of the mouse uterus during implantation and decidu-oma formation as demonstrated by changes in the distribution of alkaline phosphatase. *J. Reprod. Fert.* **8,** 331.

KIRBY, D. R. S. (1965) *The invasiveness of trophoblast.* In: The Early Conceptus, Normal and Abnormal. Ed. W. W. Park. Livingstone, Edinburgh.

KIRBY, D. R. S., BILLINGTON, W. D. & JAMES, D. A. (1966) Transplantation of eggs to the kidney and uterus of immunised mice. *Transplantation,* **4,** 713.

MCCARTHY, J. C. (1965) Genetic and environmental control of foetal and placental growth in the mouse. *Anim. Prod.* **7,** 347.

MCLAREN, A. (1965) Genetic and environmental effects on foetal and placental growth in mice. *J. Reprod. Fert.* **9,** 79.

MCLAREN, A. (1968). *Mechanisms affecting embryo development.* In: The Mammalian Oviduct. Eds. E. S. E. Hafez and R. J. Blandau. Chicago University Press.

ROBERTSON, F. W. & REEVE, E. C. R. (1955) Studies on quantitative inheritance. VIII. Further analysis of heterosis in crosses between inbred lines of *Drosophila melanogaster. Z. indukt. Abstamm.-u.VererbLehre.* **86,** 439.

SVED, J. A., REED, T. E. & BODMER, W. F. (1967) The number of balanced polymorphisms that can be maintained in a natural population. *Genetics, Princeton,* **55,** 469.