Embryonic death at implantation after induction of dominant lethal mutations in spermatids and spermatozoa of the mouse

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One of the effects of ionizing radiations on male and female germ cells is the induction of dominant lethal mutations (Bateman, 1958a, 1959a, b; Parker, 1959; Mandl, 1964). Gametes carrying such a mutation are capable of fertilization but the zygote is not viable. Little is known about the nature of the genetic change, although major chromosomal abnormalities are thought to be implicated (Bateman, 1958b, 1959a; Ehling, 1971). Some of the affected zygotes die before implantation while others survive long enough to stimulate a decidual reaction or 'mole', which persists throughout pregnancy (Bateman, 1958a, 1959a). Since earlier studies of embryonic death in the peri-implantation period have relied upon examination of implantation sites at 8.5–12.5 days *post coitum* (*p.c.*), the precise stage at which death occurs has not yet been determined. The present report is of a detailed histological examination of implantation sites at 6.5 days *p.c.* in female mice mated to X-irradiated males (i) to see whether there was a consistent pattern of degenerative change in embryos which died at or around the time of implantation and were presumed to carry a dominant lethal mutation; (ii) to define the stage at which death occurred; and (iii) to compare the decidual response in implantation sites containing normal and degenerating conceptuses. As a result of the findings, implantation sites at 5.5 days *p.c.* were also studied.

Testes of mice were exposed to 550 rads X-rays, a dose known to induce a high incidence of dominant lethal mutations in spermatozoa and spermatids (Bateman, 1958a). Gametes irradiated as epididymal and testicular spermatozoa were sampled in matings which took place in the 2nd week after irradiation and spermatids in the 3rd week (Bateman, 1958a).

Initial studies

Five 8-week-old male and ten 10–15-week-old female mice of the LACA strain were used. Four males were anaesthetized and a dose of 550 rads (300 kV, 5.0 mA, 103 rads/min) was delivered to the scrotal region, the rest of the body being shielded by 6 mm lead. One male was anaesthetized and served as an unirradiated control. At the beginning of Week 2 (i.e. 7 days after irradiation) each of the four irradiated males was caged with one female: this female was removed and replaced by another for the duration of Week 3. Two females were housed with the control male throughout Weeks 2 and 3. The morning on which a copulation plug was found was designated Day 0.5 of pregnancy (*p.c.*) All females were killed at 6.5 days *p.c.* The reproductive tract was exposed and the number and position of decidual swellings recorded. The uterus was removed intact, pinned onto stiff card and placed in Bouin's aqueous solution for 24 hr: the ovaries were also dissected out and placed in fixative. Serial sections of the uteri (8 μ m thick) and ovaries (10 μ m thick) were prepared by standard histological procedures.

Sections of the uteri were scanned in sequence ($\times 20$) and the contents of each decidual swelling examined in detail ($\times 320$): embryos were classified as normal (according to the description of Theiler, 1972) or degenerating. An estimate of the amount of decidual tissue surrounding each implantation chamber was made for 10 normal and 10 abnormal implantation sites, using an eyepiece graticule. The thickness of the decidual swelling was measured from the margin of the implantation chamber to the external surface of the uterus, in a plane at right angles to an axis formed by the mesometrium and the centre of the uterine crypt, in the section judged to pass through the central region of the

conceptus and/or implantation site. The sections of the ovaries were also scanned and the number of CL was recorded.

All the females mated and functional CL were found. Although the experiment was not designed to provide quantitative information on embryonic loss, the observations were consistent with previous findings (e.g. Bateman, 1958a). They showed that losses during the preimplantation period (i.e. the number of ovulations, represented by the number of CL less the number of implantation sites) and around the time of implantation were higher in females mated to irradiated males than in those mated to controls. The mean number of ovulations per female was 11.5 (range 7–15). The number of implantation sites in each of the two females mated to unirradiated males was 12, compared with 7.5 (6–9) and 5.8 (1–9) in females mating in Weeks 2 and 3 respectively, after irradiation of the male. Seventy-seven implantation sites at 6.5 days *p.c.* were examined microscopically. All of those in females mated to control males were normal, whereas there were 17 abnormal implantation sites in females mating in the 2nd week and 11 in females mating in the 3rd week after irradiation of the male.

Embryos classified as normal (Pl. 1, Fig. 1) showed a prominent ectoplacental cone, a pro-amniotic cavity and embryonic tissues clearly differentiated into ectoderm and endoderm. Cells of the peripheral trophoblast were flattened, the uterine epithelium had disappeared and a few primary trophoblastic giant cells were seen amongst cells of the uterine stroma, where considerable decidual transformation had taken place. Implantation sites classified as abnormal were consistently similar in appearance (Pl. 1, Fig. 2). The embryonic tissues were completely disorganized and it was difficult to distinguish cells of embryonic origin amongst the cellular debris in the implantation chamber. Although the uterine epithelium was absent there was no evidence that trophoblastic giant cells had invaded the decidual tissue. A decidual response had been initiated, however, and it was qualitatively similar to that associated with a normal conceptus.

The control male mated with one female in Week 2 and the other in Week 3. An analysis of variance was carried out independently on measurements obtained from normal implantation sites and abnormal implantation sites in order to examine their homogeneity between Weeks 2 and 3. Results showed that differences between control groups were not significant ($P > 0.2$) and differences between experimental groups were also not significant ($P > 0.2$). Hence measurements obtained from the two control females were pooled for comparison with the pooled experimental data. The mean value for the thickness of decidual tissue surrounding normal and degenerating embryos was 0.75 ± 0.01 (S.E.) mm and 0.53 ± 0.01 mm, respectively. Results of an unpaired *t* test carried out on these measurements showed that the difference between the means was statistically significant ($P < 0.001$) and hence the smaller size of the decidual swellings surrounding abnormal implantation sites could not be ascribed to sampling peculiarities.

It is clear that the zygote degenerated before 6.5 days *p.c.* and it is suggested that these embryos, which are presumed to carry a dominant lethal mutation from the male gamete, have a common histological phenotype.

Further study

The results of the initial study led to the hypothesis that the zygote survives to between 4.5 and 5.0 days *p.c.* To test this, irradiated males were mated as before and implantation sites were examined at 5.5 days *p.c.* Of the sites in the experimental group, 41 were classified as normal and 45 as abnormal; 19 of the abnormal sites were in females which mated in Week 2 and 26 in females which mated in Week 3. All but one of the conceptuses in the control group were normal. Each normal blastocyst had expanded and ectoplacental cone, ectoderm and endoderm could be distinguished. Trophoblast cells appeared closely adherent to cells of the uterine stroma and the uterine epithelium had disappeared (see Theiler, 1972). In every abnormal implantation site the blastocyst had either failed to expand or collapsed. Cells of the embryonic region were not differentiated and degenerative changes were visible, in some cases markedly advanced. Nevertheless it was always possible to identify the blastocyst even though its structure was abnormal. Parts of the uterine epithelium had disappeared and in these regions some trophoblast cells were flattened and in contact with the uterine stroma.

PLATE I

Implantation sites in mice autopsied at 6.5 days *p.c.* **Fig. 1** shows a normal conceptus and **Fig. 2** an abnormal implantation site. All sections were cut in a transverse plane at right angles to the long axis of the uterus; the mesometrial border lies beyond the left of each figure. DCR, decidual cell reaction; EC, ectoplacental cone; PC, proamniotic cavity; EP, embryonic plate; CD, cellular debris. $\times 90$.

Conclusions

In the normal process of implantation, trophoblast cells first form a close association with cells of the uterine epithelium before invading the epithelial cell layer which degenerates (Smith & Wilson, 1974). The attachment process is thought to trigger the decidual cell reaction (Finn, 1965). Observations of abnormal implantation sites at 6.5 days *p.c.* showed that the trophoblast had induced a decidual cell reaction. The amount of decidual tissue was less than that surrounding normal embryos at 6.5 days *p.c.*, suggesting that the decidual stimulus was transitory. Observations at 5.5 days *p.c.* revealed that the trophoblast had begun to differentiate and invade the uterine epithelium but indicated that the blastocyst had begun to degenerate before 5.0 days *p.c.* (about 115 hr *p.c.* according to Theiler, 1972).

Embryonic death at implantation following the induction of dominant lethal mutations in the male gamete can therefore be ascribed to failure of the blastocyst at an early stage of its differentiation. The cause of death at implantation probably differs from that in the preimplantation period, believed to be due to abnormal nuclear division (Brenneke, 1937).

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