tional source of antigenic stimulation. The fact that trophoblast may be protected by a pericellular mucoprotein need not necessarily preclude the recognition of at least some of its antigens by the maternal organism.

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The Egg and Immunology

Interest in the immunological aspects of pregnancy has to a great extent been limited to the period of placentation when the embryo is firmly 'grafted' on to the mother. Is there reason to believe that immunological events play a role earlier in pregnancy, at fertilization, pre-implantation, and implantation itself? First one must consider whether the mammalian egg is antigenic and thus capable of entering into an immunological reaction. Sell, Coombs and Edwards (*see* Gardner & Edwards 1968) were unable to detect transplantation antigens on the unimplanted mouse blastocyst. Olds (1968), on the other hand, has claimed that H_2 antigens can be detected on the surface of the mouse egg at the morula stage of development. Michie & Anderson (1966) found that in the rat sperm preferentially united with ova of a dissimilar genotype and concluded that this result constituted direct evidence of immune involvement even as early as fertilization. There can be no doubt that the mouse blastocyst expresses transplantation antigens at the time of implantation. The blastocyst can be readily and selectively destroyed at this time by standard immunological procedure (Simmons & Russell 1965, Kirby *et al.* 1966).

If the mouse egg is antigenic why is it not damaged during its residence in the uterus of an immunologically hostile mother? Simmons & Russell (1967*a*, *b*) have recently postulated that the unimplanted egg is protected from an immune attack by a non-cellular barrier, the zona pellucida. They found that when transplanted to an extrauterine site in an immunized host allogeneic mouse eggs sometimes survived. They speculated that in these unexpected successful transplants the zona pellucida had persisted around the egg until the trophoblast had formed. Shelesnyak *et al.* (1967) also consider that the shedding of the zona pellucida exposes the antigens of the blastocyst.

During normal (non-delayed) pregnancy immunization of the mother against the paternal strain antigens has no deleterious effect on the offspring, indeed there is some evidence that such procedures are beneficial (for review *see* Kirby 1968). I have recently sought to determine whether any increase in mortality of embryos could be demonstrated by prolonging the zona-free existence of blastocysts in the uteri of highly immunized mice (Kirby 1969*b*).

The mice used in the experiments were from the C57BL and C3H strains, which are strongly histo-incompatible with one another. Females of the C57BL were highly immunized against the C3H by repeated spleen cell injection and skin grafts. The C57BL recipient females were then bilaterally ovariectomized and received into the uterus either C3H or A2G (control) blastocysts. The mice were then maintained on progesterone (2 mg/day subcutaneously). It is now well established that this process leads quickly to shedding of the zona pellucida and the firm attachment of the zona-free blastocyst to the wall of the uterus (Fig 1). Fourteen days after the blastocysts had been transplanted the host mice were administered œstradiol (0·04 µg subcutaneously) to induce any remaining blastocysts to implant. Four days later the mice were killed and the uterus inspected for embryos. Of the 12 C57BL blastocysts transferred 5 implanted, and of the 12 A2G control blastocysts transferred 6 implanted.

It is clear that the prolonged existence of zona-free mouse blastocysts in an immunologically hostile uterus does not cause their death. Cytotoxic antibodies appear not to harm the rabbit fœtus, and there is no evidence that lymphocytes are able to gain access to the denuded blastocysts. None have been found in the uteri at this time, nor do the lymphocytes appear to insinuate themselves between the cells of the uterine

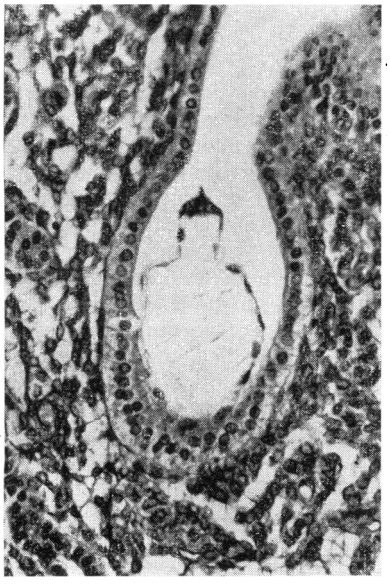

Fig 1 *Mouse blastocyst not enveloped by a zona pellucida firmly attached to the wall of the uterus during experimental delayed implantation. The mouse was bilaterally ovariectomized and maintained on progesterone*

epithelium, to which the blastocyst is firmly adherent.

Do immunological events play a role at the time of implantation? I have reviewed this subject recently at some length (Kirby 1969*a*) and will therefore consider the problem only briefly here. There is no doubt that implantation of the blastocyst is a hazardous time, and considerably more eggs are shed than implant. For example Boshier (1968) has recently demonstrated that in the mouse, depending on the strain, between 1 in 4 and 1 in 9 of the eggs ovulated fail to implant. Moreover, in the mouse nearly all the eggs are fertilized (Braden 1957). Is immunological dissimilarity between blastocyst and mother an advantage or disadvantage at this time? The answer seems to be that immunological dissimilarity is an advantage in that blastocysts genetically dissimilar to the mother are more readily implanted than eggs which are genetically similar.

Clarke & Kirby (1966) have recently drawn attention to some results published by Hull (1964) in which he mated 2 inbred strains of mice, each with a characteristic coat colour. He found that litters were deficient in young whose coat colour genotype resembled the mother. As the overall litter size did not vary he suggested that selection had taken place, at or before implantation. Hull pointed out that this deficiency in offspring may not be the result of the action of coat colour genes themselves but of the action of loci sufficiently closely linked to remain associated with them through several meiotic divisions. It is significant that the H_3 histocompatibility locus is closely linked with the locus which controls the coat colour of one strain. However, the explanation of Hull's results must account for his finding that there is a deficiency of heterozygous offspring from heterozygous mothers. There are two possibilities; either there is antigenic recognition by the fœtus as well as by the mother, or the heterozygous young suffer a disadvantage unrelated to their immunological status – a disadvantage which is overcome when the mother can react against them. Results obtained by Fekete (1947) in her studies on transplantation of eggs between different strains of mice showed that the uterus is more likely to implant those eggs which are antigenically similar to the recipient strain than those in which no diversity exists.

In addition there is very recent evidence that histocompatibility also influences implantation in the rat (Silvers & Wilson, personal communication; Palm 1969).

Can these immunological reactions at implantation influence the sex-ratio of the offspring?

Y-linked Antigens

The X and Y sex chromosomes control the production of particular antigens. Those antigens linked to the non-pairing part of the Y chromosome will, of course, be possessed only by the male. These antigens can be of sufficient strength to bring about rejection of skin grafts as shown by the experiments of Eichwald & Silmser (1955). They found that in some strains of mice isografts of male skin were rejected by virgin female recipients.

If the blastocyst expresses its Y-linked antigen, male blastocysts would be marginally more dissimilar to the mother and hence *ex hypothesi* more likely to be implanted successfully than female blastocysts. Available data suggest that this may be true. In humans, for example, the world's sex ratio at birth is 0·5146 (i.e. 51·46% males). The sex ratio early in pregnancy is, however, much higher. Estimates from sex chromatin studies on large samples of induced abortions range from 0·56 to 0·70 for the first 16 weeks of pregnancy. If these figures were extrapolated back to implantation even higher values would be suggested at this time. Assuming (which may be unjustified) that there is not an early episode of female deaths this means that

either significantly more male zygotes are produced at fertilization and that losses before implantation are randomly distributed between the two sexes, or that similar numbers of male and female zygotes are formed but that more female blastocysts fail to become implanted.

Investigations of the sex chromatin or chromosomes of unimplanted blastocysts suggest that immediately before implantation there are equal numbers of male and female blastocysts in the mouse (49 males, 49 females: Vickers 1967), in the pig (38 males, 39 females: McFeely 1967) and in the rabbit (28 males, 27 females: Melander 1962) but, surprisingly, not in the hamster (63 males, 35 females: Sundell 1962). It would seem, then, that selection occurs at implantation and the male Y-linked-antigen-bearing blastocysts are preferentially implanted.

Further support for this hypothesis comes from sex ratio data in inbreeding communities. Inbreeding should tend to reduce the difference in autosomal antigens between mother and blastocyst and hence accentuate the importance of Y-linked antigens at implantation. Studies of cousin marriages (Kirby *et al.* 1967) show that 420 males and 343 females were born – a sex ratio of 0·55. They also showed that if there had already been consanguinity within three generations of the first cousin marriage the sex ratio rose to 0·61.

ABO blood group antigen compatibility between mother and offspring has recently been related to placental size in humans (Jones 1968). Presumably compatibility affects trophoblast activity. Certainly A antigen at least has been demonstrated on the surface of human trophoblast cells (Gross 1966). If at implantation mother and offspring are compatible on the ABO blood group system (because the mother does not possess the specific antibody) then Y-linked antigen should become more conspicuous on the blastocyst. It follows that in these groups more male blastocysts will implant with a resultant elevation of the sex ratio at birth. The data available (Allan 1959) support this hypothesis. AB mothers (who possess neither anti-A nor anti-B antibody) have significantly more male than female babies (sex ratio of 0·55). Similarly there is a high sex ratio of O babies of O mothers, and B babies of B mothers. A babies from A mothers, however, have a low sex ratio. This exception cannot be explained. If the babies are of blood group O (no A or B antigens) they should have *ex hypothesi* a higher sex ratio quite irrespective of the blood group of the mother. Again the evidence is confirmatory (Allan 1959).

Conclusion

The data on which these hypotheses are based are often small, inadequately controlled or awaiting confirmation. When more investigators direct their attention to this early period of pregnancy and some of the confused issues are sorted out we shall know with more certainty whether immunological events play a role before the placenta is formed.

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The Conceptus as an Allograft: Immunological Reactivity of the Mother

Immunological isolation of the mammalian fœtus probably plays an important role in maintaining its freedom from rejection by the mother. It is unlikely, however, that such isolation can be the sole mechanism responsible for ensuring the successful development of an allogeneic conceptus. The existence of the fœto-maternal barrier (Currie 1968) remains in doubt, especially in view of the disturbing and unlikely results of Tuffrey *et al.* (1969).

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