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Germ Cells in Natural and Experimental Chimeras in Mammals

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Germ cells in natural and experimental chimeras in mammals

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Introductory remarks

Chimerism and its origin

A chimera can be defined as an individual composed of cells derived from two or more separate zygotes. The incorporation of the two cell lines into the embryo can occur spontaneously or be produced experimentally. It can take place at various periods of embryogenesis and in various ways. It seems that in mammals three mechanisms can be involved (see Tarkowski 1969 for a detailed discussion and references).

Double fertilization combined with immediate cleavage of the oocyte at the first or second meiotic division

Both dispermy and immediate cleavage have been described but simultaneous occurrence of both events in one egg has not, as yet, been observed, nor experimentally reproduced.

Fusion of cleaving embryos

The technique of fusing of mouse embryos *in vitro* was developed by the present author (Tarkowski 1961) and by Mintz (1962, 1964) and proved to be a very efficient tool in producing chimeric individuals. (As recently shown by Gardner (1968) chimerism can also be produced by injection of single cells to blastocysts). However, it remains to be shown whether or not fusion of cleaving embryos can occur spontaneously *in vivo*.

These two mechanisms share many features in common—the two cell lines co-exist in the embryo from the very beginning of embryogenesis and their initial contribution to the embryo is equal. As a result, the cells of both types are able to contribute to most, if not all, somatic tissues, as well as to the germinal tissue. Chimerism originating with the help of either of these two mechanisms will be referred to as 'primary'.

Interchange of cells between dizygotic twins via vascular anastomoses

This often occurs in cattle and marmosets and sporadically in other mammals, including human beings. It operates, however, at a much later stage of embryogenesis and, consequently, the number of cell types which can be affected by such an exchange is limited. The occurrence of chimerism in the hemopoietic tissue has been well established, but conflicting reports have been presented regarding chimerism in the non-vascular tissues and in the germinal tissue (for references see Tarkowski 1969; also p. 110). In order to emphasize the different origin and nature of this particular type of chimerism it will be referred to as 'secondary'.

Each of the three mechanisms described above can produce individuals composed of cells of either similar or dissimilar sex. The former are unlikely to be discovered unless special genetic or chromosomal markers are involved.

Origin of germ cells

It is now generally accepted that in mammals primordial germ cells originate in the hind region of the embryo and/or in the surrounding extra-embryonic tissues and migrate along the hindgut and the mesentery to the genital ridges (for references, see Franchi, Mandl & Zuckerman (1962)). The evidence that these cells are the only precursors of the definite germ cells is now quite conclusive. Other points remain unclear, however, e.g. what is the initial number of these cells and whether their formation coincides or precedes the time when they acquire characteristic morphological and/or cytochemical features.

In 1962 Ohno, Trujillo, Stenius, Christian & Teplitz suggested that in the bovine embryo primordial germ cells might also migrate through the vascular route (see p. 110). However, according to a detailed embryological study by Jost & Prepin (1966), and contrary to an earlier report by Ohno & Groop (1965), very few cells displaying features of primary germ cells can be found in the blood vessels of the bovine embryo at the time of migration. It seems, therefore, that the migration of these cells via the blood vessels is an accidental rather than regular event, if it occurs at all.

2. Germ cells in chimeras

Primary chimerism

Chimeras composed of cells of similar genetic sex

No doubt, the majority of chimeras of spontaneous origin that are composed of cells of the same genetic sex remain undetected, unless the differences between the two genotypes express themselves clearly phenotypically, e.g. in pigmentation. The only chimera of this type described so far, was a female mouse (Russell & Woodiel 1966), which displayed chimerism in both the coat and in the germinal tissue. A number of such animals have been recently produced in the mouse with the help of the technique of embryo fusion.

In a group of nine mice produced by fusion of CBA-p and CBA-T6T6 embryos, the two strains differing in their pigmentation and karyotype, Mystkowska & Tarkowski (1968) discovered two males and one female composed of like-sexed components. When back crossed to CBA-p animals, all three chimeras produced two types of offspring, thus providing evidence of the heterogenous composition of the germinal tissue. The contribution of the two cell lines to the germinal tissue and to the bone marrow, the only somatic tissue in which the composition was quantitatively estimated, was not strictly alike, but did not differ by more than 30 %.

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Mice displaying both somatic and germ-cell chimerism were also produced by Mintz (1968). This author found also that in chimeric males of strain combination C3Hf and C57BL/6, there was a selection against germ cells of C57BL/6 genotype. In these particular animals selection appears to operate postnatally in the diploid phase of spermatogenesis. It is quite likely that selection can occur at any stage of the life-history of germ cells, starting with the pregonadal period, and that it can vary depending on the combination of genotypes which were employed in chimera formation.

Chimeras composed of cells of dissimilar genetic sex

The individuals displaying this form of chimerism present undoubtedly a far more interesting experimental model. Some of these individuals at least can be expected to contain in their gonads both genetically male and female somatic cells, and at least originally, germ cells of both genetic sexes as well. Studies carried out in our laboratory (Tarkowski 1961, 1963, 1964; Mystkowska & Tarkowski, 1968, 1970) and also those made by other authors (Mintz 1968; McLaren & Bowman 1969) show conclusively that in mice produced by fusion of cleaving embryos true hermaphroditism is a very rare event, and that the majority of the animals undergo normal sexual differentiation to become phenotypically normal males or females. The incidence of hermaphroditism and the sex ratio of the offspring both seem to depend on the genotypes of the embryos that are fused together. In some combinations of genotypes all sex chromosome chimeras appear to develop into males or hermaphrodites; in others the sex ratio approaches a 1:1 ratio with some of these animals developing into females as well. Leaving this question aside, the fact remains that in a substantial part of these chimeras germ cells of one genetic sex are likely to enter into the gonadal primordium which will subsequently differentiate in the opposite direction. How do they behave in this environment?

Among CBA-p/CBA-T6T6 chimeras Mystkowska & Tarkowski (1968) discovered two chimeric fertile males. Although they contained as much as 19 and 51 % of XX cells in bone marrow, both produced spermatozoa only from the genetically male component. Moreover, XX germ cells were absent among primary spermatocytes in diakinesis-metaphase I. These observations suggest that the cells in question were either eliminated from spermatogonial population early in life, or that they survived as spermatogonia but degenerated during the early phases of meiotic prophase. It is noteworthy that also in the hermaphrodite, in which genetically female cells predominated in the coat and composed up to 97 % of the bone marrow, all primary spermatocytes were of XY constitution.

These data are substantiated by observations made by Mintz (1968), who is also of the opinion that in chimeric animals gametes are always derived from the cells in which the genetic sex corresponds to the phenotypic sex. In other words, the course of gametogenesis cannot be reversed. Although this generalization probably holds true both for XX and XY germ cells, data about the fate of genetically male germ cells in the ovarian tissue are not yet conclusive.

Although several chimeras of spontaneous origin containing cells of dissimilar genetic sex have been described in mammals, including men (for references, see Tarkowski (1969)), in only one case have the germ cells been investigated karyologically in the testes. In a tricoloured XX/XY cat studied by Malouf, Benirschke & Hoefnagel (1967) all primary spermatocytes in metaphase I proved to be of XY constitution. This finding confirms the observations made on experimentally produced mouse chimeras.

The absence in these males of spermatozoa and of primary spermatocytes derived from XX

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germ cells raises questions as to when and how these cells are eliminated. Karyological preparations from testes of such adult chimeric males yielded practically no spermatogonial mitotic plates and therefore it was not possible to ascertain whether or not XX germ cells could survive as spermatogonia until adulthood (Mystkowska & Tarkowski 1968). This question remains in fact still unresolved, but there are some observations on the behaviour of germ cells in the testicular tissue of chimeric males which are relevant to the problem under consideration.

In our studies on chimeras of CBA-p/CBA-T6T6 origin (Mystkowska & Tarkowski 1968) we described an example of oocytes developing in the otherwise normal testis of a 5-day-old male. Although the animal was not examined karyologically, it seems more than likely that it was a sex chromosome chimera. This observation encouraged us to examine more closely the gonads of embryos in the 16th and 17th day of pregnancy and of early postnatal chimeras (Mystkowska & Tarkowski 1970). In none of the twelve males aged 8 to 20 days were oocytes encountered in their testes. However, in five out of eleven male embryos germ cells in meiotic prophase were found side by side with normal pre-spermatogonia. Three of these embryos were examined karyologically and proved to contain in their livers both XX and XY cells. It seems safe, therefore, to postulate that the presence of meiotic germ cells in embryonic testes, which is an unusual event to occur in males prenatally, is connected with the sex chromosome chimerism of these individuals. However, since the genetic sex of these cells remains unknown, it is not possible to say which one of the following two alternatives is true: (i) The meiotic germ cells are of XX constitution; they have started meiotic prophase on the time-schedule characteristic of females, irrespective of the testicular environment. (ii) The cells in question can be of either genetic sex and the initiation of meiosis was conditioned by some environmental factors peculiar to the testis of an XX/XY individual. It can be suggested that because of the co-existence in the somatic gonadal tissue of XX and XY cells, the environment provided by such a testis differs in some way from the environment provided by a normal testis. This interpretation inevitably implies that only a part of XX germ cells present in the embryonic testis enter into meiotic prophase. What happens to the others? One of the possible explanations is that they behave in the last days of embryonic life as typical pre-spermatogonia, thus surviving, at least temporarily, beyond birth (see also, Tarkowski 1970; Mystkowska & Tarkowski 1970). Further studies along these lines are being continued in our laboratory.

Secondary chimerism

Ohno *et al.* (1962) presented karyological evidence for the presence of XX cells in the testes of bulls born as a co-twin to a female. According to these authors, the XX cells belonged to a germ line rather than to a somatic line. This finding was recently confirmed in three bulls by Teplitz, Moon & Basrur (1967), who in each of the three bulls investigated encountered in addition a few meiotic plates in which the characteristic XY bivalent was lacking and which therefore were identified as being of XX constitution. Similar observations were reported by Benirschke & Brownhill (1963) in chimeric male marmosets composed of cells of dissimilar genetic sex. The conclusion inferred from all these observations was that primordial germ cells can migrate from one twin to another. While the mitotic metaphases with XX sex chromosomes could have originated from cells other than germ cells, the presence of meiotic configurations is more convincing evidence, assuming that the bivalents have not been misinterpreted. However, these observations have not been confirmed in the more recent studies by Dunn, Kenney, Stone & Bendel (1968) and Evans, West & Ford (personal communication) who

were not able to discover either mitotic or meiotic XX metaphase plates in the testes of bulls born from heterosexual pregnancies. Weiss & Hoffmann (1969) described recently chimerism of leukocytes and of the gonadal tissue in several pairs of unlike-sexed bovine twins. However, in view of the fact that chimerism of the gonads was assessed on the basis of examination of cells grown in tissue cultures, the opinion of the present author, contrary to that of Weiss & Hoffmann, is that these findings provide evidence for chimerism of the somatic tissue rather than of the germinal tissue. It is equally difficult to explain, however, how the soma of the gonads became chimeric.

Serological examination of progeny from two bulls born to freemartins (Stone, Berman, Tyler & Irvin 1960; Dunn *et al.* 1968) failed to prove that any spermatozoa had developed from alien XX germ cells. Stone *et al.* (1960) and Stone, Friedman & Fregin (1964) also tested the possibility of germ cell exchange between like-sexed twins and formation of gametes from alien germ cells. Serological investigations of such two animals and their progeny did not provide evidence in favour of such a possibility. Thus the evidence for exchange of primordial germ cells between dizygotic twins is clearly in conflict and the whole problem awaits further investigations.

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