The purpose of the present study was to trace the fate of primordial germ cells in mouse chimeras of XX/XY constitution. In this type of research one hopes to obtain knowledge of the role of intrinsic genetic factors and of environmental factors (the environment provided by the gonads) in initiating and directing the course of gametogenesis in mammals.

Data obtained up to the present show that adult males with sex chromosome chimerism produce spermatozoa only from the genetically male component and that in these individuals XX germ cells are not present among primary spermatocytes in diakinesis (Mystkowska & Tarkowski, 1968). The first of these observations has recently been confirmed by Mintz (1968). Since chimeras formed of components of the same genetic sex can produce gametes of both 'parental' genetic types, it seems likely that, in XX/XY individuals also, primordial germ cells of both types are formed and populate the genital ridges, and that the absence of XX germ cells in adult XX/XY males is secondary rather than primary. The finding of growing oocytes existing with spermatogonia in the testis of a 5-day-old male chimera (Mystkowska & Tarkowski, 1968) and an earlier observation (Tarkowski, 1964) of the co-occurrence of germ cells in meiosis and spermatogonia in the testicular tissue of newborn hermaphrodite chimeras, directed our observations to the late embryonic and early postnatal period. We decided to concentrate on the 16–17th day of embryonic development, when in normal development germ cells in females are in meiotic prophase, and in males in resting phase, and on the 8–20th day of postnatal development.

1 Author’s address: Research Institute of Mother and Child Health, Karowa 2, Warsaw, Poland.
2 Author’s address: Department of Embryology, University of Warsaw, Warsaw 64, Poland.
MATERIALS AND METHODS

The material consists of 40 individuals obtained by fusing two 8–16-cell embryos of (1) A strain (6 individuals), (2) non-inbred albino mice (27 individuals) or (3) A/CBA F₁ hybrids backcrossed to A (7 individuals). The two embryos used for fusion always came from the same female and in the majority of cases from induced ovulation (5 i.u. PMSG followed in 36–42 h by 5 i.u. HCG: Gestyl and Pregnyl, 'Organon'). The methods used differed from those previously described (Mystkowska & Tarkowski, 1968) only in that Mulnard culture medium (Mulnard, 1967) was used instead of Brinster medium. Mulnard medium radically improved the development of the embryos.

The recipients were non-inbred albino females mated with CBA vasectomized males. Eggs were transferred to the uterus on the evening of the third day or on the morning of the fourth day, taking the day on which the vaginal plug was found as the first.

Nine pregnant recipients were killed on the 16th and 17th days of pregnancy. Two females gave birth, and four were killed on the 20th or 21st day of pregnancy, the embryos removed by Caesarian section and placed with foster mothers which had littered 2–4 days earlier. All 19 newborn mice survived the immediate postnatal period, but four young died on the 8th, 9th and 15th day of life. The remaining young were killed either between the eighth and tenth day or between the 15 and 20th day of life.

The gonads were fixed in Bouin’s fluid, embedded in paraffin wax, sectioned serially at 6 μ and stained with Ehrlich’s haematoxylin and eosin. Each section of the embryonic testes was examined for cells undergoing meiosis. To assess the percentage of these cells the total numbers of prespermatogonia and of meiotic cells were counted on every fifth section, separately for each testis.

Chromosome preparations from the liver of embryos killed on the 16th and 17th day of embryonic life were made by Ford & Woollam’s method (1963).

RESULTS

A. Embryos on 16–17th day of development

21 embryos: 10 females and 11 males.

Females. Upon histological examination the ovaries proved to be completely normal, with all germ cells in meiotic prophase, the majority in the pachytene stage.

Males. Cells in meiotic prophase were found in the testes of 5 out of 11 males, in addition to the typical prespermatogonia (Fig. 1). In four of these individuals the testes were of normal structure, but in one some regions of the testes were atypical: there were no clearly differentiated sex cords and the majority of the germ cells were in meiotic prophase (Fig. 2). This is the only example in the whole of the present material which could possibly be considered a true
Table 1. Co-occurrence of XX and XY cells in the liver, and of prespermatogonia and meiotic cells in the testes of chimeric male embryos

<table>
<thead>
<tr>
<th>No. of embryo</th>
<th>Total no. of cells</th>
<th>Liver</th>
<th>Testes</th>
<th>Right testis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>XX</td>
<td>Left testis</td>
<td>No. of germ cells examined</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>Not investigated</td>
<td>—</td>
<td>3345</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0</td>
<td>2459</td>
<td>134</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>13</td>
<td>1492</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>27</td>
<td>2076</td>
<td>67</td>
</tr>
<tr>
<td>8</td>
<td>Not investigated</td>
<td>—</td>
<td>2218</td>
<td>856</td>
</tr>
</tbody>
</table>

Three additional males had no meiotic cells in the testes and were not investigated karyologically.
Sexual differentiation in chimeras

hermaphrodite. Macroscopically the genital system was typically male, but as the sex ducts were not histologically examined we cannot exclude the possibility that the Müllerian ducts were present in a rudimentary form.

The number of cells in meiotic prophase differed greatly in different individuals but in all cases was far smaller than the number of prespermatogonia (Table 1). In two cases the percentage of meiotic cells in the two gonads of each individual was similar, and in two others the differences were considerable (0·7:16·3 % and 9·7:38·6 %). In the fifth embryo (no. 4 in the Table) there were only three meiotic germ cells found in a total count of over three thousand. Meiotic cells were often grouped within gonads, but almost always germ cells typical of the normal testis at this period of development existed in the same area.

The six remaining males had completely normal gonads, with no trace of cells in meiotic prophase.

Chromosomal studies on the liver. Nine of the 21 embryos were examined karyologically; with one exception a minimum number of 20 metaphase plates was scored. Of the five males with cells in meiosis in the testes, three were examined karyologically and all, as might have been expected, were sex chromosome chimeras (Table 1). XX/XY chimerism was also found in one male which had no cells in meiotic prophase in its testes.

Three phenotypically normal female embryos were karyologically examined; the results were as follows: (1) 20 metaphase plates examined: all XX; (2) 64 metaphase plates examined: 53 XX, 11 XY; (3) 5 metaphase plates examined: 4 XX, 1 XY (Fig. 4).

B. Individuals at 8–20 days of postnatal life

19 individuals: 7 females and 12 males.

Females. The ovaries of six animals were similar in appearance to the ovaries of normal animals of the same age. However, in one female which died 15 days after birth one ovary contained structures resembling sterile seminiferous tubules situated in the region of the hilus (Fig. 3). The genital tract of this female did not display, at least macroscopically, any abnormalities.

Males. In all males the testes were normal and did not contain either growing

---

Fig. 1. Section through testis of chimera (no. 6 in Table 1), 17th day of embryonic development. Prespermatogonia and cells in meiotic prophase side by side in sex cords: the short and long arrows respectively indicate one example of each. × 1000.

Fig. 2. Section through testis of another chimera (No. 8 in Table 1), 16th day of embryonic development, showing area without clearly defined sex cords. The majority of germ cells are in meiotic prophase. × 600.

Fig. 3. Section of ovary of a 15-day-old female chimera. Structures resembling sterile seminiferous tubules are present in the region of the hilus. × 120.

Fig. 4. Genetically male metaphase plate from the liver of a chimeric female, 16th day of embryonic development. Long arrow = Y chromosome; short arrows = two smallest autosomes. × 1200.
oocytes or other cells (living or degenerating) which might have originated from cells entering meiosis before birth. Individuals in this group were not karyologically examined but in view of the results of chromosomal examination of the embryos it seems reasonable to assume that there were sex chromosome chimeras among the 12 males.

C. Sex ratio

The overall sex ratio in our present material was 23 ♂:17 ♀ (57.5:42.5%) and the predominance of males was thus not great. If one male embryo were considered a hermaphrodite (see above), the frequency of occurrence of hermaphroditism would still be at an exceptionally low level in the present sample (1/40 = 2.5%). However, since the embryos used for producing chimeric individuals were of three different genetic types (see Materials and Methods), the overall sex ratio in such a heterogeneous sample seems to us of doubtful significance. Only one of the three groups of chimeras (non-inbred albino) was large enough for calculating the sex ratio, which was 17♂:11 ♀.

DISCUSSION

A. Sex differentiation of chimeras

Data on the sex ratio obtained up to the present time in our laboratory (Tarkowski, 1961, 1963; Mystkowska & Tarkowski, 1968) pointed to a distinct predominance of males and a relatively rare occurrence of hermaphrodites, while the frequency of occurrence of females varied around the theoretical frequency, that is, about 25%. A predominantly male sex ratio has also been reported recently by McLaren & Bowman (1969). The suggestion of Tarkowski (1961) that the majority of XX/XY chimeras develop into phenotypically normal males, has been confirmed by karyological investigations (Mystkowska & Tarkowski, 1968). The present data differ somewhat from previous data. The absence of hermaphrodites in the relatively large sample of 40 individuals is remarkable, but what is particularly striking is that males only slightly out-number females. The assumption that this results from development of XX/XY embryos into females as well as males, has been confirmed karyologically in the present study by discovering two XX/XY phenotypically normal female embryos. Mintz (1965) also reported ‘a very distinct preponderance of males’ but in a recent report (Mintz, 1968) recorded only 1.3% of hermaphrodites and a very slight predominance of males over females (52.0–46.7%) in material comprising over 400 individuals. Unfortunately, this author does not present the data in the various genotype combinations, which could be even more interesting than the otherwise impressive number of animals produced. In our opinion differences in the sex ratio between various series with various genetic combinations can be meaningful, and they should be emphasized and not effaced. This may form a starting point for more subtle analysis of the mechanism of sex differentiation.
Sexual differentiation in chimeras

of chimeras: for example, genetic factors other than those involving the sex chromosomes directly may have to be taken into account.

Irrespective of deviations in different series, it is an undoubted fact that individuals of XX/XY constitution most often 'regulate' sex differentiation in the direction of one or the other sex, and relatively rarely form both types of gonadal tissue to become hermaphrodites. It would seem that although in different genetic combinations the pattern may vary somewhat, the dominating direction of differentiation is to the male phenotype; the development of the female phenotype may perhaps require the marked predominance of genetically female cells.

It should be borne in mind, however, that, up to the present, karyological identification of sex chromosome chimerism rests solely upon studies of tissues other than the gonads, usually just one somatic tissue, or at best two tissues (e.g. bone marrow—Mystkowska & Tarkowski, 1968; Mintz, 1968; liver—present study; kidney and bone marrow—Mintz, 1968). The lack of comparable data on the composition of different tissues in single chimeric individuals means that one cannot with any certainty accept the data for a single tissue as a general criterion. Local differences in the various parts of the body or even within one organ may always occur and an individual example could be misleading if taken as a standard.

This is especially important when one deals with sexual differentiation. Leaving aside the exceptional cases, as, for instance, testicular feminization syndrome, it is generally accepted that the sex phenotype develops in accordance with the phenotype of the gonads, and that the development of sex ducts on each side is under the local control of each gonad. The gonadal tissue (somatic rather than germinal) appears to be the only tissue in the reproductive tract which is directly shaped by the intrinsic sex-determining factors, all other events involved in sexual differentiation coming as the result of the primary activity of the gonads. The composition of the gonadal primordia of an XX/XY chimera (and probably the spatial distribution of both cell types within the rudiments) is therefore decisive for the whole course of sex differentiation. While such data are not available at present, observations made on spontaneous sex chromosome chimeras and mosaics (cf. Tarkowski, 1969, for discussion and references) show that testes can develop normally despite the fact that they contain a substantial number of genetically female cells. It seems that this is also so of the majority of XX/XY mouse chimeric males.

There are no such data for ovaries, except for the observations of Gartler, Waxman & Giblett (1962), who found XY cells present in the ovarian tissue of an ovotestis of a human hermaphrodite.

B. Germ cells

Interpretation of the observations requires in the first place an answer to a fundamental question, namely, are germ cells of both genotypes (irrespective of
their sex chromosome composition) originally present in the gonadal rudiment of a chimera? Our previous studies showed that where the genetic sex of the two components was identical, the population of germ cells was mixed and quantitative relations in the somatic and germinal tissue did not differ by more than about 30% (Mystkowska & Tarkowski, 1968). It would therefore appear that unless one component strongly dominates over the other, primordial germ cells of both genotypes penetrate into the genital ridges. Original quantitative differences may result from variations in the composition of the embryonic tissue in different places, including the site of differentiation of these cells, and also perhaps from internal genetic factors responsible for their proliferation during the pregonadal period.

There are no a priori data to indicate that the original population of primordial germ cells in the gonads of XX/XY chimeras meet different conditions from those in like-sexed chimeras. It seems therefore likely that XX and XY primordial germ cells are originally present in the gonadal primordia (again with the exception of certain extreme cases).

The discovery of a few oocytes in the growth phase in the normal testis of a 5-day-old chimera (Mystkowska & Tarkowski, 1968) indicated that: (1) in the testes of that, and presumably of other, individuals some of the germ cells began meiosis before birth, at a time typical of females, and (2) at least some of these cells do not degenerate and do not undergo first reduction division, but begin the female type of gametogenesis. The present studies have in fact shown that some of the germ cells in the testes enter into meiotic prophase, at a time when this phenomenon is observed in ovaries. These cells occur only in males which are sex chromosome chimeras, although not in all individuals. As in the case of oocytes described in the previous report (Mystkowska & Tarkowski, 1968), the genetic sex of these cells is unknown. The simplest explanation would be the assumption that these are XX cells which are fulfilling their destiny. However, this explanation is premature, and is not easy to reconcile with the following facts. In the first place the percentage of meiotic cells in the total population of germ cells is usually low, most often far lower than the percentage of XX cells in liver. In the second place the frequency of their occurrence in the two testes of one individual can be very different (see Table 1). Differential migration of primordial germ cells to the gonads seems very unlikely. According to an alternative interpretation, the entry of a given cell into meiotic prophase during the final phase of embryonic development is not connected with its genetic sex but is conditioned by the local conditions prevailing at that place in the testis. In XX/XY chimeras the somatic tissue of the testis most probably consists of cells of the two genetic sexes, differently distributed. Although the histogenesis of the testis as a whole follows a normal course, the existence of a differentiated environment for germ cells is more than likely. The initiation of meiosis as the result of environmental factors would also explain the small number of cells in meiosis—since the gonad as a whole differentiates as a testis—and differences in the number of these cells
Sexual differentiation in chimeras

in the two testes. Tarkowski (1970) recently put forward a hypothesis according to which both XX and XY germ cells are potentially capable of entering meiosis prenatally, at the time when this normally takes place in females. However, in normal males, XY germ cells are subject to the inhibitory action of the somatic tissue of the testis, expressed inter alia by inhibition of, or marked reduction in, mitotic divisions. Disturbance of the normal differentiation of the testis following transplantation of the early genital ridge appears to affect this inhibiting action, making it possible for some germ cells to begin meiosis at a time characteristic of females (W. Ożdżeński, in preparation). It has also been suggested (Tarkowski, 1970) that the initiation of the meiotic prophase prenatally is the expression of an 'internal clock' of the germ cells, rather than the result of stimulation by factors extrinsic to these cells.

In the light of data obtained up to the present it would seem that the majority of the cells which begin meiosis in the testicular tissue before birth degenerate soon afterwards and only a few enter the growth phase. Because of their environment, these too sooner or later degenerate. The present study did not provide a single example of the formation of oocytes, as was found once in a CBA-p/CBA T6T6 chimera, despite the fact that the absolute number of meiotic cells in the testes of chimeras before birth is fairly high. Thus oocytes either form very rarely, or degenerate very rapidly, not surviving to the 20th, and probably not even until the 10th, day of postnatal life.

In our previous work it was shown that in the testicular tissue of adult XX/XY individuals, XX germ cells are not represented among primary spermatocytes in the diakinesis stage, suggesting that the female genetic constitution does not permit cells to pass through spermatogenesis. This conclusion finds support in the genetic studies of Mintz (1968) on mouse chimeras and also in the observations made by Evans, Ford & Searle (1969) who failed to find any XO cells among primary spermatocytes in an XO/XYY mosaic mouse. However, neither the above studies nor the present study have established whether all XX germ cells are eliminated early in development or whether some become spermatogonia but do not attain diakinesis, degenerating during the course of meiotic prophase.

SUMMARY

1. Gonads of 40 individuals produced by fusion of two cleaving embryos were examined histologically. These comprised 21 16–17-day embryos and 19 8–20-day-old mice. The liver of nine embryos was investigated for sex chromosome chimerism.

2. The sex-ratio in the present sample, comprising three different genetic types of chimeras, was $23\,\text{♂}:17\,\text{♀}$.

3. Two phenotypically normal female embryos, out of three karyologically examined, showed XX/XY chimerism.

4. In 5 out of 11 male embryos, the testes contained germ cells in meiotic
prophase, as well as prespermatogonia. Three of these embryos were karyologically examined and proved to be XX/XY chimeras. An additional XX/XY male had no meiotic germ cells.

5. None of the 12 males examined postnatally contained growing oocytes in the testes, suggesting that most meiotic germ cells degenerate at the end of prophase.

6. The genetic sex of the meiotic germ cells present in the embryonic testes remains unknown, but the indirect evidence suggests that the prenatal initiation of meiosis is governed in chimeras by environmental conditions rather than by intrinsic genetic factors. The present study leaves open the question of whether all XX germ cells in XX/XY chimeras are eliminated soon after birth, or whether some survive as spermatogonia but degenerate as soon as they enter into meiosis.

RÉSUMÉ

Le comportement des cellules germinales et la différenciation sexuelle dans les chimères de souris aux stades embryonnaires avancés et après la naissance

1. Des gonades de 40 individus résultant de la fusion de deux embryons en cours de segmentation ont été soumis à l'examen histologique. Parmi ceux-ci, il y avait 21 embryons de 16 à 17 jours et 19 souriceaux de 8 à 20 jours. Les chimères relatives aux chromosomes sexuels ont été recherchées dans le foie.

2. La sex ratio, qui correspondait à trois types génétiques différents dans le cas présent, était de 23 ♂ et de 17 ♀.

3. Deux embryons femelles, à phénotype normal, ont montré un chimérisme XX/XY sur trois cas examinés au point de vue caryologique.

4. Dans 5 sur 11 embryons mâles, les testicules contenaient des cellules germinales en prophase méiotique, en même temps que des préspermatogonies. Trois de ces embryons ont été examinés au point de vue caryologique et furent constates comme étant des chimères XX/XY. Un autre mâle XX/XY ne présentait pas de cellules germinales en méiose.

5. Aucun des 12 mâles examinés après la naissance ne contient d'ovocytes en croissance dans ses testicules, ce qui fait penser que la plupart des cellules germinales en méiose dégénèrent à la fin de la prophase.

6. Le sexe génétique des cellules germinales méiotiques présentes dans les testicules embryonnaires est inconnu, mais on a une preuve indirecte que le déclenchement de la méiose chez le foetus chimère est commandé par des facteurs d'environnement plutôt que par des facteurs génétiques. La présente étude laisse ouverte la question de savoir si toutes les cellules germinales XX sont éliminées peu de temps après la naissance dans les chimères XX/XY ou si certaines d'entre elles survivent à l'état de spermatogonies mais dégénèrent sitôt après leur entrée en méiose.
REFERENCES

genetics 8, 87–96.


MINTZ, B. (1968). Hermaphroditism, sex chromosomal mosaicism and germ cell selection in

MULNARD, J. G. (1967). Analyse microcinématographique du développement de l'œuf de


857–60.


12, 735–57.


and germinal tissues of a mammalian gonad stable or labile? In Environmental Influences on
Genetic Expression, (in the Press).

(Manuscript received 12 June 1969)