Oogenesis in adult prosimians

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INTRODUCTION

It is widely accepted that oogenesis normally stops early in mammalian development (see Brambell, 1956; Franchi, Mandl & Zuckerman, 1962). Nevertheless, it has been claimed that mitotically active oogonia, and oocytes in early stages of meiotic prophase occur in mature specimens of Galago senegalensis (Gérard, 1920, 1932; Gérard & Herlant, 1953; Herlant, 1961; Petter-Rousseaux, 1962; Butler, 1964), G. crassicaudatus (Gérard & Herlant, 1953), G. demidoffi (Gérard, 1932; Gérard & Herlant, 1953; Petter-Rousseaux, 1962), Perodicticus potto (Gérard & Herlant, 1953), Loris tardigradus lydekkerianus (Rao, 1927; Brambell, 1930), and Daubentonia madagascariensis (Petter-Rousseaux & Bouriére, 1965). The latter is a lemuroid prosimian, while all the others are lorisooids (Hill, 1953). It has also been asserted that new germ cells are formed by direct transformation from the somatic cells of the ovarian germinal epithelium (Gérard, 1920, 1932; Rao, 1927; Gérard & Herlant, 1953). These claims are totally inconsistent with current ideas on ovarian development and a re-examination of ovaries from adult prosimians was undertaken to confirm or deny them.

MATERIALS AND METHODS

The material used comprised 3 complete ovaries of Perodicticus potto (2 pregnant animals), plus representative sections from 1 non-pregnant adult; 6 ovaries of Loris tardigradus lydekkerianus (2 from 1 adult animal, the rest juvenile); 9 ovaries of Galago demidoffi (7 animals); and 7 ovaries of G. crassicaudatus (7 animals).

Each of the complete ovaries was fixed in Bouin's aqueous fluid for up to 24 h, and then transferred to 70% alcohol, dehydrated and embedded in paraffin wax. Two labelled G. demidoffi ovaries were then processed for autoradiography (see below). The other ovaries were sectioned serially at 5 or 7 μ, and stained in Weigert’s iron haematoxylin and 'chromotrop 2R'.

An attempt was made to confirm the presence of any DNA-synthesizing germ cells in adult G. demidoffi by an autoradiographic technique. Two animals were given an intra-peritoneal injection of 225 or 150 μc of tritiated thymidine

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(Radio-chemical Centre, Amersham, Bucks.), and killed 24 h later. The ovaries were fixed, dehydrated and embedded as for the normal histological preparations (see above). Sections were cut at 5 μ, mounted on gelatin-treated slides, and dewaxed in the usual way. Autoradiographs were then prepared by a dipping technique using Ilford K2 emulsion, exposed for 10 or 7 days, and developed in a dilute ‘Amidol’ developer for 6 min. After fixation and washing, the slides were stained with Harris’ haematoxylin for 2 min, dehydrated and mounted in DePeX.

The exact age of the animals was unknown. Since ovarian development is related to age (see Franchi et al. 1962), an attempt was made to assess the maturity of ten of the specimens by their degree of development towards a full permanent dentition, and by the amount of epiphyseal fusion shown on X-ray photographs. The animals available for assessment in this way were 1 Loris, 2 Perodicticus, 2 G. crassicaudatus, and 5 G. demidoffi. All of these 10 animals had fully developed permanent dentition. The 2 Perodicticus had well-worn teeth, while the other animals showed little wear. The Loris and 3 G. demidoffi showed incomplete fusion of the epiphyses; all the other animals showed complete fusion.

RESULTS

Histological observations

Large nests of germ cells were found in the outer zone of the ovarian cortex of both G. crassicaudatus and Perodicticus. These nests were roughly circular in section, contained several dozens of germ cells, and were usually enclosed by a thin basement membrane (Plate 1, fig. A). Oogonia were observed at interphase (Plate 1, fig. B), and in all phases of mitosis (Plate 1, figs. B, C); oocytes at each stage of meiotic prophase were also found (Plate 1, figs. B–D). Large oocytes at diplotene were more usually seen outside the germinal nests, and enclosed in a primary follicle (Plate 1, fig. D). Degenerating germ cells were found at all stages of oogenesis.

The three complete Perodicticus ovaries examined were taken from two pregnant animals. One of these ovaries contained a small corpus luteum. The germ-cell nests were as numerous in this ovary as in those lacking a corpus luteum.

PLATE 1

Fig. A. Nest of germ cells, showing oogonia and oocytes at various stages of development: Galago crassicaudatus.
Fig. B. Oogonia at interphase (o) and at mitotic prophase (p); oocyte at zygotene (z): G. crassicaudatus.
Fig. C. Oogonium at anaphase of mitosis; oocyte at pre-leptotene: G. crassicaudatus.
Fig. D. Oocyte at diplotene: Perodicticus potto.
Fig. E. Oocytes at leptotene (l): G. demidoffi.
Qualitative observations suggested that the ovaries of *G. demidoffi* contained fewer germ-cell nests, and fewer germ cells, than those of either *G. crassicaudatus* or *Perodicticus*. Nevertheless, those cell nests which did occur contained oogonia at interphase, and oocytes at leptotene, zygotene or pachytene (Plate 1, fig. E). No signs of oogonial mitoses were found. Oocytes at diplotene occurred outside the cell nests and in primary follicles.

All stages of oogenesis were found in the juvenile *Loris* ovaries (Plate 2, figs. F, G). The adult ovaries of this species contained a number of oogonia at interphase (Plate 2, fig. H), but only a few in mitosis, usually degenerating (Plate 2, fig. H). A few oocytes in leptotene and pachytene were observed (Plate 2, fig. I), and many at diplotene. These germ cells were scattered through the ovarian cortex, and not aggregated in germ cell nests.

** Autoradiographic observations **

One ovary from each of two adult *G. demidoffi* was processed for autoradiography, and examined by both transmitted and incident illumination. A fair number of germ cells were found to be labelled, mostly oogonia at prophase of mitosis. Photographs were taken of selected fields of the ovary. The first of a pair was taken with the microscope focused on the cells (Plate 2, fig. J). The second photograph was taken by incident illumination (Plate 2, fig. K), with the objective focused on the autoradiographic label in the emulsion above the cells.

** DISCUSSION **

These results confirm that oogonia at interphase or in the various stages of mitosis occur in undoubtedly adult specimens of the two species of *Galago* examined, in *Loris*, and in *Perodicticus*. Although few signs of mitotic activity were seen in ovaries from adult *Loris*, an examination of large numbers of ovaries from mature specimens of this species has already shown that germ cells occur at all stages of mitosis (Anand Kumar, 1966). Oocytes at the successive stages of meiosis up to diplotene are also present, often in great numbers. While oogonia may occasionally be seen in the germinal epithelium, there is no reason to believe

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** PLATE 2 **

Fig. F. Oogonia at interphase (o); early mitotic prophase (p); and metaphase (m): juvenile *Loris tardigradus lydekkerianus.*

Fig. G. Oocytes at diplotene. Juvenile *Loris.*

Fig. H. Oogonium at interphase (o); atretic division (a): adult *Loris.*

Fig. I. Oocytes at leptotene (l); and pachytene (pa): adult *Loris.*

Fig. J. Oogonia at mitotic prophase: *Galago demidoffi.*

Fig. K. Autoradiographic label above field shown in fig. J, viewed by incident illumination.
that such cells are derived from transformed epithelial cells. It is more likely that all germ cells in the adult prosimian ovary are derived from daughter cells of pre-existing oogonia, as is known to be the case for all other mammals studied (see Franchi et al. 1962).

The ovaries of a few other mammals show an approach to the prosimian condition. Thus Winiwarter (1920) described early meiotic stages in the ovaries of a pre-pubertal cat. In the rabbit the entire development of oocytes takes place post-natally, the neonatal ovary containing no germ cells other than oogonia (Teplitz & Ohno, 1963; Peters, Levy & Crone, 1965). The Mongolian gerbil (Meriones unguiculatus) shows a similar condition (Ioannou, preliminary observations).

Germ cell nests in ovaries from adult animals have been described before. Hartman (1926) claimed to have observed a periodic outburst of oogenetic activity in the ovary of the post-pubertal opossum. Proliferation of germ cells was said to take place from a limited area of ‘embryonic epithelium’ on each side of the hilum, and to give rise to Pflüger’s tubes containing germ cells. Hamlett (1935) described a button of tissue at the hilar region of the ovary of an adult armadillo which, he claimed, contained oogonia and oocytes arranged in cords. In his report on the ovaries of pre- and post-pubertal armadillos, Enders (1960) described medullary cords persisting in adult ovaries. Often these cords were devoid of germ cells, but gonia and cells in meiotic prophase were reported in about 30% of the ovaries he examined. In such cases, pyknosis, karyolysis and other degenerative changes were also evident. Enders believed that these cell bodies were formed from residual granulosa cells of primary follicles.

The cell nests found in prosimian ovaries may thus be directly comparable with Pflüger’s tubes derived from proliferations of the embryonic germinal epithelium. In the prenatal animal these differentiate into the spermatic tubules in the male and the medullary cords in the female (see Franchi et al. 1962). In almost all other mammalian ovaries studied the germ cells contained in these cords pass through mitosis and the early stages of meiotic prophase by the time of birth or shortly after. Yet in prosimians the cords persist well into adulthood and contain actively dividing oogonia and oocytes.

The examination of ovaries of adult specimens of G. demidoffi by autoradiographic techniques, following intraperitoneal injection of 3H-thymidine, was undertaken to confirm the observation, in histological preparations, of early stages of meiosis. Some germ cells took up the radioactive isotope; labelled cells were diagnosed as either oogonia in mitotic prophase or oocytes at pre-leptotene or leptotene. It is not surprising that the proportion of labelled germ cells was low. Although 24 h had elapsed between injection and autopsy, the work of Rubini, Cronkite, Bond & Fliedner (1959, 1960) indicates that thymidine is available for incorporation for only 30–60 min before being degraded. Clearly, the number of cells which are synthetically active at the time the isotope is
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administered will depend on (a) the developmental condition of the ovary (see Peters, Levy & Crone, 1962), and (b) the length of the pre-mitotic and pre-meiotic synthetic periods, in the species being investigated. Thus the present results demonstrate that oogonia and oocytes in the ovaries of adult prosimians are capable of DNA synthesis. The author knows of no comparable experimental demonstration of oogenesis in mature mammals. Whether such germ cells ever constitute a part of the definitive oocyte population is another matter. This problem might be investigated by injecting $^3$H-thymidine into adult specimens, and then allowing a period of weeks or months to elapse before autopsy. Examination of autoradiographs might then demonstrate whether or not labelled germ cells go through meiotic prophase to diplotene, and subsequently become invested in primary follicles. If such cells were found it would seem reasonable to infer that neo-oogenesis in adult prosimians does indeed add to the stock of definitive germ cells.

SUMMARY

1. It has been claimed that new germ cells are formed after maturity in certain female prosimians. A histological study was made of the ovaries of adults of four of these species (Galago crassicaudatus, G. demidoffi, Perodicticus potto and Loris tardigradus lydekkerianus), and of juvenile specimens of Loris. In addition, two adult female G. demidoffi were injected with $^3$H-thymidine and their ovaries subjected to autoradiographic examination.

2. Mitotically active oogonia and/or oocytes in the early stages of meiotic prophase were present in all the adult prosimians examined; in G. demidoffi some of these cells incorporated $^3$H-thymidine, suggesting that DNA synthesis was in progress. These observations indicate that active oogenesis was taking place. There is no reason to believe, however, that new germ cells are formed other than by division of pre-existing oogonia.

RÉSUMÉ

Oogenèse chez les Prosimiens adultes

1. Selon certains auteurs de nouvelles cellules germinales se formeraient après la maturité sexuelle chez la femelle de certains Prosimiens. Une étude histologique a été faite sur les ovaires adultes de quatre espèces (Galago crassicaudatus, G. demidoffi, Perodicticus potto, Loris tardigradus lydekkerianus) et sur de jeunes spécimens de Loris. De plus, deux femelles adultes de G. demidoffi furent injectées avec de la thymidine-$^3$H et leurs ovaires furent soumis à l’examen autoradiographique.

2. Des oogonies en mitose et des oocytes aux premières étapes de la prophase méiotique ont été observées chez tous les Prosimiens adultes examinés; chez G. demidoffi, quelques unes de ces cellules ont incorporé la thymidine-$^3$H; ceci
suggère que la synthèse de DNA était en cours. Ces observations indiquent qu'une oogenèse active se déroulait. Il n'y a pas de raison, cependant, de penser que nouvelles cellules germinales se forment, autrement que par la division des oogonies préexistantes.

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REFERENCES


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