PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Migration of Gonocytes Into the Mammalian Gonad and Their Differentiation

Hannah Peters

Phil. Trans. R. Soc. Lond. B 1970 **259**, 91-101
doi: 10.1098/rstb.1970.0048

Phil. Trans. Roy. Soc. Lond. B. **259**, 91–101 (1970) [91]
Printed in Great Britain

Migration of gonocytes into the mammalian gonad and their differentiation

By Hannah Peters
The Finsen Laboratory, The Finsen Institute, Copenhagen, Denmark

(*MS. received May* 1969)

[Plates 10 and 11]

Contents

Introduction

The migration of the germ cells into the mammalian gonad and their subsequent differentiation has been the subject of many investigations and controversies. However, sufficient facts have accumulated which led Witschi (1962) to state that 'the formerly controversial subject of the origin and unbroken continuity of the germ cell lines can now be considered settled'. Histochemical techniques for alkaline phosphatase have made it possible to selectively stain and identify primordial germ cells (McKay, Hertig, Adams & Danziger 1953; Chiquoine 1954; Mintz 1959). The path and development of the germ cells has been described in detail in histological investigation (for review see Brambell 1956; Mintz 1960; Franchi, Mandl & Zuckerman 1962) and recently labelling techniques have made the continuity of the germ cell line 'visible' (Rudkin & Griech 1962; Peters, Levy & Crone 1962; Kennelly & Foote 1966; Borum 1966; Peters & Crone 1967).

Migratory phase of germ cells

Brambell (1927) recognized the germ cells in the early germinal ridges of the mouse embryo. Since then their path has been identified. The germ cells arise extra-gonadally in the yolk sac and migrate via the hindgut endoderm to the mesentery of the gut, to the mesonephric folds and finally enter the germinal ridges (Witschi 1948; Everett 1943). The primitive germ cells actively migrate by amoeboid movement (Blandau, White & Rumery 1963).

In the mouse, primordial germ cells can be recognized in the early 8-day embryo. Two days later the first germ cells arrive inside the germinal ridges. On the 12th day of embryonic life, migration is completed and the germ cells are concentrated within the gonad (Chiquoine 1954; Mintz 1960).

While on the migratory path the germ cells multiply: in 8-day-old mouse embryos their number is 100 to 150, one day later they number 400 to 500 (Chiquoine 1954). By the time the migratory period of the germ cells is ended about 5000 of them populate the gonad (Mintz & Russell 1957).

The gonads of a 12-day-old mouse embryo contain germ cells which are characteristic and distinguishable from the epithelial cells. However, a morphological difference between male and female germ cells is not yet evident (figure 1, plate 10). It is only towards the end of the 12th day that the organization of the gonad (not the appearance of the germ cells) indicates differentiation between the sexes (Simkins 1923; Brambell 1927). Usually the differentiation of the female gonad lags somewhat behind that of the male, so that a female gonad is often first identified by the absence of formation of seminiferous cords already present in male litter mates.

The germ cells in both sexes divide mitotically at this age, and are morphologically similar as seen under the light microscope. Electron microscopy reveals that the development of the oogonial endoplasmatic reticulum is more advanced than that in the gonocyte (Franchi & Mandl 1964).

Differentiation of the male gonad

The testis becomes recognizable by the formation of the sex cords which are limited from the surrounding tissue by a fine basal membrane. The tunica albuginea and the interstitial tissue begin to form. The sex cords contain two kinds of cells, the larger ones are identified as gonocytes, the smaller ones as supporting cells (Hargitt 1926; Clermont & Perey 1957). Mitoses among the gonocytes as well as among the supporting cells are numerous. A separation of the cells takes place (on day 15 in the mouse and day 16 in the rat) when the supporting cells become arranged in palisade-like fashion close to the basement membrane, while the gonocytes come to lie in the centre of the cord where they are easily recognized, not only because of their position but also by their characteristic appearance (figure 2, plate 10). Division and multiplication in the gonocytes do not continue during the whole of the embryonic period but are halted at a certain stage of embryonic development (on day 14 in the mouse and day 18 in the rat). The non-proliferating phase of the gonocyte continues into the neonatal period. Soon after birth the gonocytes migrate from their central position towards the periphery of the seminiferous cord, where they make contact, through cytoplasmatic processes with the basement membrane and resume mitotic activity (Franchi & Mandl 1964; Novi & Saba 1968) (figure 3, plate 10). Alinement on the basement membrane appears to be a necessary prerequisite for further differentiation and division of the germ cells (Nebel, Amarose & Hackett 1961).

Not all gonocytes present in the testis at birth survive, for at least 50 % of them are lost during the first week of life (Beaumont & Mandl 1963; Roosen-Runge & Leik 1968; Huckins 1963). Those that survive begin to divide. The resumption of division coincides with the appearance of spermatogonia which lead to the conclusion that the gonocyte is the direct precursor of the spermatogonia (Clermont & Perey 1957; Sapsford 1962*a*, *b*; Beaumont & Mandl 1963). Hilscher & Makosi (1968) propose that the actual spermatogenesis begins when the gonocyte resumes division, giving rise to two daughter cells which are different from the parent cell. This division is thought to be unique.

Meiosis begins soon after the resumption of mitosis and with it the recurring waves of spermatogenesis (Clermont & Bustos-Obregon 1968; Courot 1962). The time table for the beginning of this process varies in different species. In the mouse the first meiotic cycle begins at the age of 8 to 10 days (Nebel *et al.* 1961), in the rat it starts 3 to 4 days later (Clermont & Perey 1957). In the ram the normal rhythm of spermatogenesis is initiated after the first cells in meiosis appear in the 75-day-old animal (Courot 1962).

DNA synthesis in the gonocyte during embryonic life has been investigated in the mouse

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

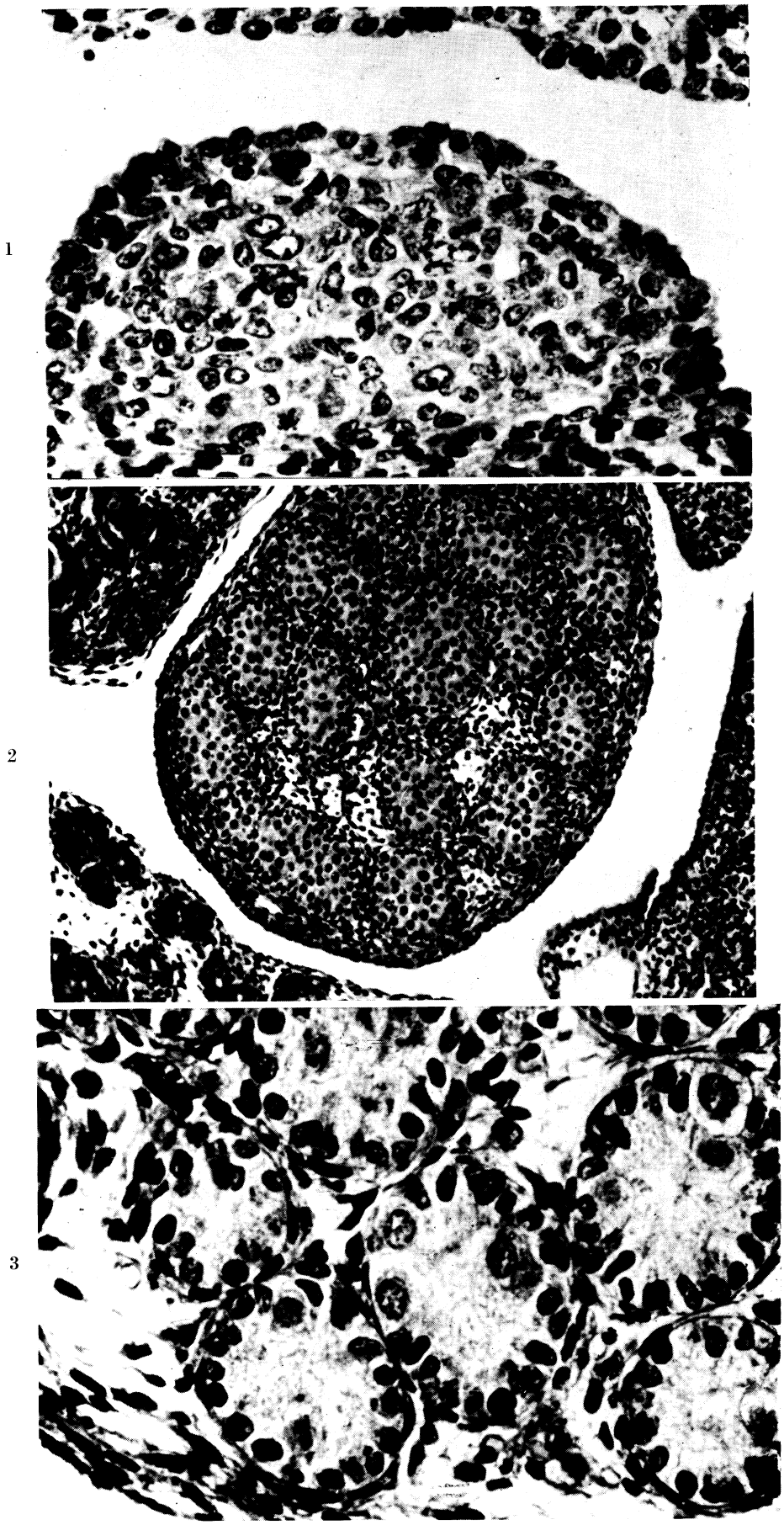


FIGURE 1. Undifferentiated gonad in a 12-day-old mouse embryo (magn. × 510).

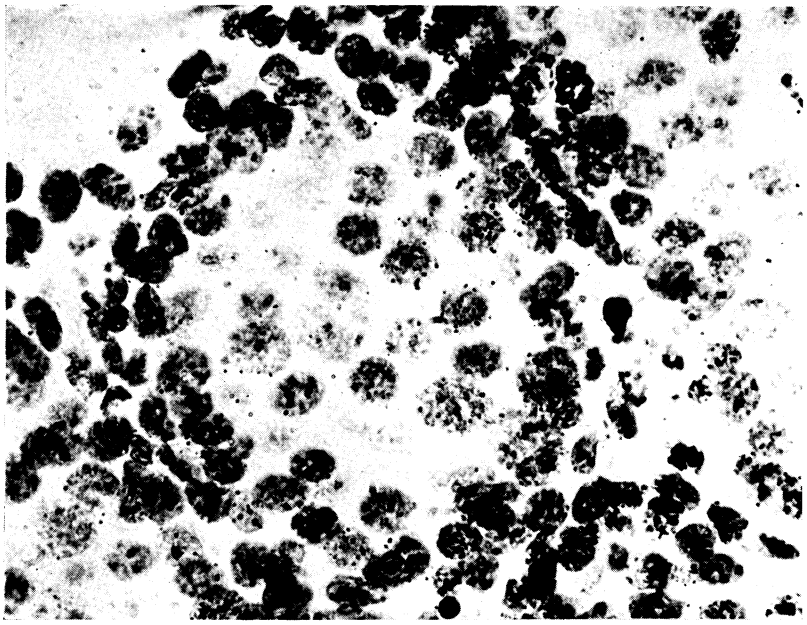
FIGURE 2. Testis of a 16-day-old mouse embryo. Gonocytes lie in the centre of the seminiferous cord (magn. × 153).

FIGURE 3. Testis of a newborn mouse. Some gonocytes are seen lying close to the basement membrane in the seminiferous cords (magn. × 510).

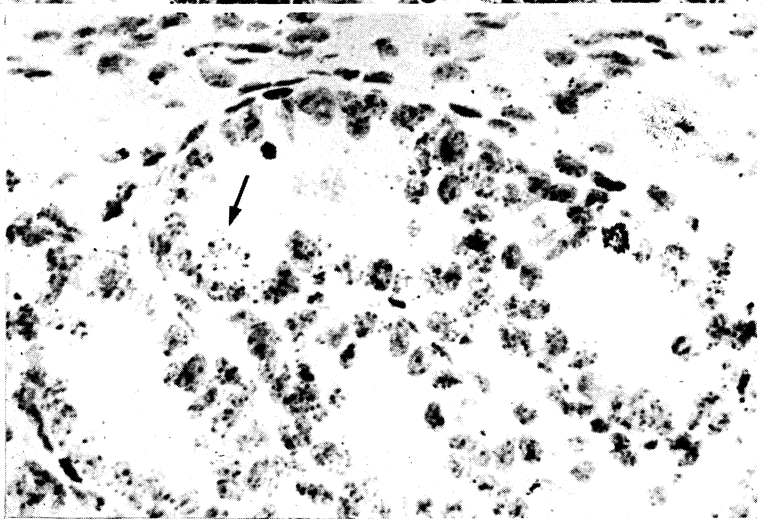
(*Facing p.* 92)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

4



5



8

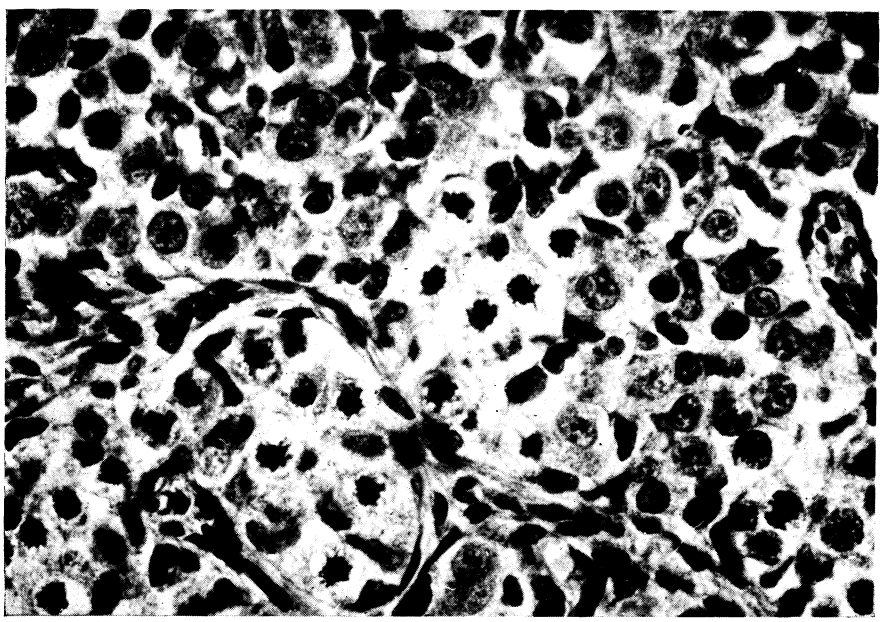


FIGURE 4. Labelled gonocytes in the testis of a 15-day-old embryo that had received ^{3}HTdR 2 days earlier (magn. × 540).

FIGURE 5. Labelled gonocyte (↑) seen in the autoradiograph of the testis of a 3-day-old mouse that had received ^{3}HTdR on the 13th day of embryonic life (magn. × 210).

FIGURE 8. Ovary of a 4-day-old rabbit with groups of oogonia and groups of oocytes in early stages of meiotic prophase (magn. × 510).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(H. Peters, unpublished) and during the neonatal period in the rat (Hilscher & Makosi 1968). Injection of tritiated thymidine (^{3}HTdR) into mouse embryos between the ages of 12 and 18 days resulted in the labelling of gonocytes only when the label was injected before day 14 (figure 4, plate 11). The abrupt cessation of incorporation of the label into the gonocyte nucleus is in marked contrast to the behaviour of the supporting cells, which continue to incorporate the radioactive DNA precursor on all days during the last trimester of pregnancy (table 1). This is consistent with the histological observation that gonocytes do not divide while supporting cells continue division after the 14th day of embryonic life.

TABLE 1. LABELLING OF GONOCYTES AND SUPPORTING CELLS AFTER INJECTION OF ^{3}HTdR AT DIFFERENT AGES DURING EMBRYONIC LIFE (MOUSE)

animal	age of embryo at time of injection (days)	time interval between injection and sacrifice (hours)	percentage of labelled cells	
			gonocytes	supporting cells
1	12	48	14	35
2	12	48	12	not determined
3	13	48	40	37
4	13	48	38	37
5	14	2	0.6	32
6	14	24	0	40
7	14	6	0.4	not determined
8	15	24	0	48
9	18	2	0	45

Some gonocytes which populated the seminiferous tubules already before the 14th day of embryonic life persist in the neonatal period, as can be shown by injecting ^{3}HTdR into a mouse on the 13th day of pregnancy and preparing an autoradiograph of the testis of her offspring a few days after birth (figure 5, plate 11). Labelled gonocytes were found in such autoradiographs. Some were still lying medial to the supporting cells, others had already migrated to the basement membrane. These labelled cells make the continuity of the germ cells from foetal life to the neonatal period 'visible', as the cells carrying the label in the neonatal period must be identical with, or have directly arisen from, gonocytes synthesizing DNA at the time of the injection of precursor during embryonic life.

DIFFERENTIATION OF THE FEMALE GONAD

In the female also the ultimate germ cell, i.e. the egg, is a direct descendant of the primordial germ cell that migrated into the gonad at some stage of embryonic development. The germ cells in the female gonad, the oogonia, divide mitotically giving rise to daughter cells. At a certain point of the development, however, mitosis in these cells is halted and the germ-cell population does not increase any further. The cells enter meiotic prophase and are subsequently called oocytes. Prophase in the oocyte is a singularly long stage in the development of this cell. In all other cells of the body prophase is completed within hours while in the oocyte it lasts for weeks, months or even years. It comes to completion only shortly before the egg is ovulated. During the long prophase the oocyte goes through various stages which cytologically are distinguishable and are defined by their chromosomal arrangement. The duration of each of the stages—

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

leptotene, zygotene, pachytene and diplotene—varies; they become progressively longer as development progresses. Leptotene is the shortest, it lasts 3 to 6 h in the mouse oocyte. Zygotene is completed within 12 to 14 h (Crone, Levy & Peters 1965). Pachytene lasts more than 60 h, while diplotene, and its modification dictyate, may continue for many years in some species.

Oogenesis, i.e. the transformation of oogonia to oocytes, occurs at a certain time during the development of the animal. Its onset and completion may lie entirely within the foetal period, entirely within the neonatal period, or begin in the foetus and come to completion during the neonatal period. In general it can be stated that oogenesis is limited to a well-defined period in the animals life. Only in certain species of the family Lemuroida are persistent islands of oogonia found which differentiate into oocytes in the ovary of the mature animal, suggesting that oogenesis continues during adult life (Herlant 1960; Brambell 1956; Petter-Rousseaux & Boulière 1965; Kumar 1968). Of the animals so far investigated (table 2) the following complete

TABLE 2. TIME AND DURATION OF OOGENESIS IN DIFFERENT MAMMALS

	oogenesis					ovulation
	onset		end		duration	
	age of foetus (days)	postpartum age (days)	age of foetus (days)	postpartum age (days)		
rat	17	.	18	.	2 days	spontaneous
mouse	13	.	16	.	4 days	
guinea-pig	30	.	50	.	3 weeks	
pig	30	.	100	.	10 weeks	
cow	75	.	160	.	12 weeks	
monkey	3 months	.	about the time of birth	.	3 months	
human	2 months	.	7 months	.	5 months	
golden hamster	.	day of birth	.	6	7 days	
rabbit	.	1	.	10	10 days	induced
ferret	.	6	.	14 to 20	8 to 14 days	
mink	.	.	.	+	.	
cat	40 to 50	.	.	8	18 days	

oogenesis during foetal life: rat (Beaumont & Mandl 1962), mouse (Brambell 1927; Borum 1961), guinea-pig (Ioannou 1964), cow (Erickson 1966), sheep (Mauleon 1961), pig (Black & Erickson 1968), monkey (Baker 1966), and human (Witschi 1948; Baker 1963; Baker & Franchi 1967). Some animals begin and complete oogenesis entirely within the neonatal period: rabbit (von Winiwarter 1901; Teplitz & Ohno 1963; Peters, Levy & Crone 1965), ferret (Deanesly 1970) and golden hamster (Weakley 1967; Greenwald & Peppler 1968). In the cat oogenesis is initiated during foetal life and continues into the neonatal period (von Winiwarter & Saimont 1909).

As an example of oogenesis which lies entirely within the foetal period, I would like to discuss briefly the situation in the mouse. Oogenesis in the mouse begins usually on the 13th day of embryonic life. On this day most of the germ cells are oogonia, many of them are seen in division. In some cells, however, meiotic prophase has begun and groups of cells in leptotene are recognized (figure 6). Thus the beginning of oogenesis in the mouse occurs on day 13 of embryonic life. On the 14th day slightly less than half of the germ cells are oogonia; they still divide and many cells are seen in mitosis next to others which are already in early stages of prophase. On the 15th day most germ cells (93 %) have already entered meiotic prophase but the oocytes are not all in the same stage of differentiation: some are in leptotene, most are in

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

zygotene and a few oocytes have already developed to pachytene. On day 16 only a few cells (2 %) are still recognized as oogonia, indicating that the transformation of oogonia to oocytes is coming to a close on this day. After the 16th day of embryonic life, oogonia are not found any more in the ovary of the mouse neither prenatally nor postnatally. Thus in the mouse, oogenesis is in progress for 4 days, as the first cells become oocytes on day 13 and the last ones on day 16 (Peters *et al.* 1962; Borum 1966).

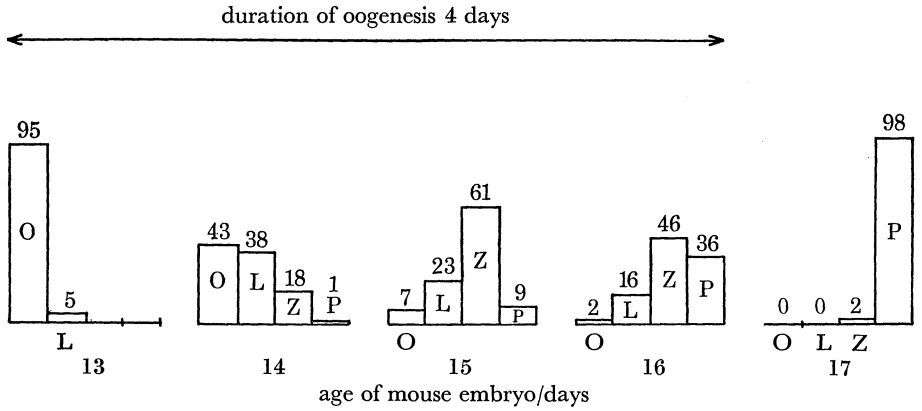


FIGURE 6. Oogenesis and stages of meiotic prophase in the mouse embryo (percentage of cells). O, oogonia; L, leptotene; Z, zygotene; P, pachytene.

During the remainder of foetal life, the oocytes develop progressively through the transitorial stages of meiotic prophase. A daily shift in the distribution of the transitory stages of meiotic prophase is seen. On day 16 oocytes in pachytene and zygotene are found in approximately equal numbers. On the following day mainly oocytes in pachytene are seen. When the animal is born on day 19 or 20 of pregnancy the ovary contains oocytes in pachytene and early diplotene.

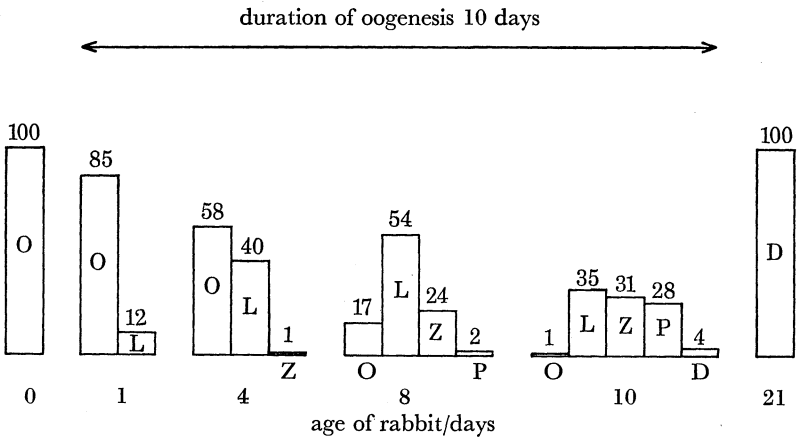


FIGURE 7. Oogenesis and stages of meiotic prophase in the neonatal rabbit (percentage of cells). O, L, Z, P as in figure 6; D, diplotene.

The actual distribution of the different transitorial stages in the oocytes at the time of birth varies in different strains of mice and is characteristic for the strain. In some strains most oocytes are already in early diplotene, in others pachytene oocytes still predominate.

The oocytes complete the transitory stages of meiotic prophase within the first 5 days after birth and thereafter remain in dictyate, the stationary phase of meiotic prophase in the mouse, until shortly before ovulation when prophase is quickly completed.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

In the rabbit oogenesis takes place entirely during the neonatal period (figure 7). On the day of birth the germ cells in the rabbit are all oogonia and many mitoses are found among them. Oogenesis begins on day 1, and continues until the animal is 10 days old.

In some cells meiosis begins on day 1, when leptotene is first seen. Oocytes in zygotene begin to appear on day 4. On that day more than half of the germ cells are still mitotically dividing oogonia. Four days later differentiation has progressed and less than one quarter of the germ cells are still oogonia, slightly more than half are oocytes in leptotene and the remainder are in zygotene. The first oocytes in pachytene are seen on this day. The last oogonia are seen when the animal is 10 days old. Thus oogenesis in the rabbit begins on day 1 and ends on day 10. Only when the animal is three weeks old are all oocytes found in the resting stage of meiotic prophase.

The duration of oogenesis

The time that elapses between the transformation of the first and last oogonia into oocytes, i.e. the duration of oogenesis, varies considerably in different species.

Oogenesis in the mammal is not a synchronized process (table 2). That it was considered to be so was perhaps because the detailed investigation of the process of oogenesis was first done in the rat, which seems to have the shortest duration of oogenesis. In this species, all oogonia have been transformed to oocytes within 2 days (Beaumont & Mandl 1962). In the mouse oogenesis continues for 4 days (Peters, Levy & Crone 1962; Borum 1966). The guinea-pig (Ioannou 1964) and the pig (Black & Erickson 1968) need 3 and 10 weeks respectively to complete the process. In the monkey (Baker 1966) and the cow (Erickson 1966) there are about 3 months between the beginning and the end of oogenesis, while in the human female (Baker 1963) oogenesis is in process for 5 months. The three species that have been found so far in which oogenesis takes place in the neonatal period are the golden hamster (Weakley 1967), the rabbit (von Winiwarter 1901; Teplitz & Ohno 1963) and the ferret (Deanesly 1969). These complete oogenesis in 1 to 2 weeks. In the mink, oogenesis is in progress during the neonatal period (H. Peters, unpublished), but the actual duration of oogenesis has not yet been established.

The range of the duration of oogenesis is rather wide, varying from 2 days to 5 months in the species so far investigated. The factors determining the duration of oogenesis are not yet identified. Its duration certainly cannot be correlated with the length of the pregnancy. The golden hamster, with an extremely short embryonic life of only 2 weeks, needs half of this period to complete its oogenesis, while the rat, with an embryonic life of 3 weeks, completes oogenesis in only 2 days. Furthermore, it is as yet difficult to predict the species in which oogenesis takes place during the embryonic period, and in which it is delayed until the neonatal period. It appears that in most species where ovulation occurs spontaneously oogenesis is completed during foetal life (an exception is the golden hamster), while in those where ovulation occurs after stimulation all or part of oogenesis takes place during the neonatal period. The question arises whether oogenesis during embryonic life might be correlated to the subsequent cyclic release of gonadotrophin in the mature animal, the delay of oogenesis until the neonatal period preventing the programming of the brain for cyclic release. Information on this question is not yet available.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Differentiation of oocytes in groups

The transformation of oogonia into oocytes is not a synchronized process but is spread over a defined period of time, although the germ cells differentiate and mature in groups (figure 8, plate 11). In a 4-day-old rabbit, for example, oogenesis is still in progress, and groups of oogonia lie close to other groups consisting of oocytes which are already in either leptotene or in zygotene, i.e. in one of the transitorial stages of meiotic prophase. That oogonia and oocytes develop in groups seems to be a general phenomenon as it has been described in the mouse (Borum 1961), rabbit (Gondos & Zamboni 1967), golden hamster (Weakley 1967), rat (Franchi & Mandl 1962), guinea-pig (Ioannou 1964), monkey (Baker 1966) and man (Baker 1963; Van Wagenen & Simpson 1965).

Differentiation on groups is also the rule in the spermatogenetic line. In histological sections of the germinal epithelium of the mammalian testis, spermatids occur in clusters consisting of eight or sixteen cells and all of the cells in such a group develop into spermatozoa at the same rate. Electromicroscopic studies of spermatogenesis (Fawcett, Susumo & Slautterback 1959) revealed that the cells in such a group are not only in close proximity but are actually connected by intercellular bridges. Such bridges connecting male germ cells lying in groups have been found in the rat, guinea-pig, hamster, rabbit, monkey and man (Fawcett *et al.* 1959). Recently intercellular bridges have also been shown to exist between oogonia and between oocytes in the

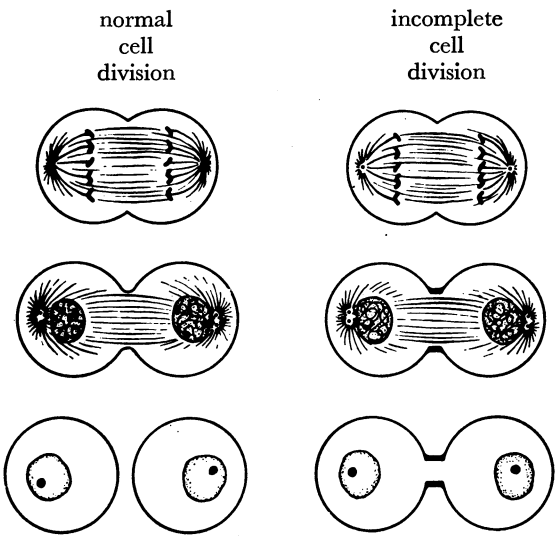


Figure 9. Diagrammatic representation of mitosis with complete (left) and incomplete (right) separation of daughter cells. (From Gondos & Zamboni 1969, by permission of the editor of *Fertility and sterility*.)

rabbit (Zamboni & Gondos 1968; Gondos & Zamboni 1969). The intercellular bridges seem essential for the coordination of the differentiation of the germ cells. The development of intercellular bridges is thought to be the result of a sequence of mitotic divisions in which the cells do not completely separate (figure 9). In complete cell division, the constriction between the daughter cells, the mid-body, remains intact only for a short time then subsequently disappears and the two daughter cells become separate entities. In incomplete cell division, as seen in dividing oogonia for example, the connexion between the daughter cells persists. It is suggested that the simultaneous mitotic division of two connected oogonia would lead to the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

formation of a group of four interconnected oogonia. The presence of organelles in the cytoplasm of the intercellular connexions suggests that material and information can be exchanged between the cells connected by bridges (Fawcett 1961; Gondos & Zamboni 1969). Cells in such a group enter meiosis simultaneously and apparently go through the different stages of meiotic prophase at the same pace. As the signal for the beginning of oogenesis does not reach all oogonia at the same time, the possible communication through intercellular bridges would satisfactorily explain the fact that different cell nests appear in different stages of development, while the individual cells within a cell nest are in identical stages of development.

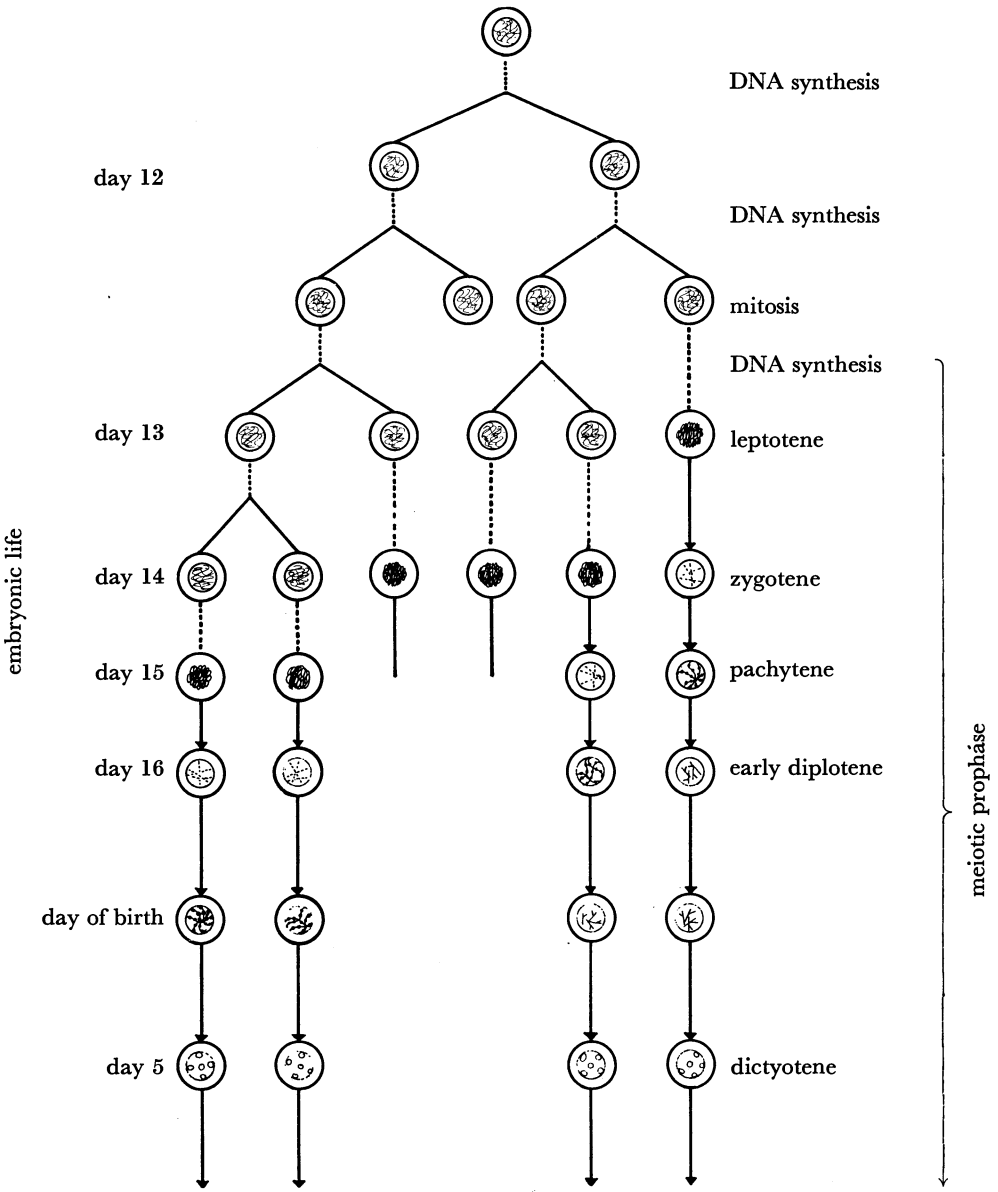


FIGURE 10. Schematic representation of oogenesis in the mouse embryo.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

DNA synthesis in oocytes

It is of interest to find out at what point of the development of the oocyte DNA synthesis takes place (figure 10). In all mitotically dividing cells DNA is synthesized during the interphase of the cell cycle. It was therefore reasonable to assume that the DNA which becomes part of the oocyte and the mature egg is synthesized in the interphase which just precedes meiotic prophase, i.e. it must lie within the period during which oogonia are transformed to oocytes. That this is actually so, has been shown in the mouse and in the rabbit (Peters *et al.* 1962; Lima-de-Faria & Borum 1962; Rudkin & Griech 1962; Crone *et al.* 1965; Peters & Crone 1967). DNA synthesis has been followed in autoradiographs after the injection of ^{3}HTdR. Injection of the radioactive DNA precursor into the mouse any time between day 13 and 16 of embryonic life resulted in labelled oocytes. Injection at any later time, prenatally, postnatally and during any time of the oestrous cycle, failed to produce labelled oocytes (Peters & Levy 1966; Borum 1966). In the rabbit labelled oocytes were only seen after injection of ^{3}HTdR between day 1 and 10 postnatally. It can therefore be concluded that DNA synthesis in the oocyte takes place in the last premeiotic interphase, whether this lies in embryonic life or in the neonatal period.

Furthermore, multiple injections of a radioactive DNA precursor within the time of oogenesis resulted in almost 100 % labelling of all surviving oocytes in the adult animal (Borum 1966; Kennelly & Foote 1966), confirming the suggestion that oocytes formed during oogenesis persist in the adult animal.

Degeneration of germ cells

Not all germ cells present in the ovary of the embryo survive. The highest number present in the female gonad occurs some time during the period when oogonia are transformed into oocytes. After mitotic division ceases the number of oocytes does not remain constant but a considerable number degenerate during the early stages of oocyte differentiation.

In the human ovary three distinct waves of atresia have been recorded affecting oogonia undergoing mitosis, oocytes in pachytene and oocytes in diplotene (Baker 1963). The rate of germ-cell degeneration varies considerably in different species. In the human (Baker 1963) and in the cow (Erickson 1966) only 5 % of the peak number of germ cells survive in the gonad at birth, 13 % survive in the monkey (Baker 1966) and the guinea-pig (Ioannou 1964); 30 % survive in the newborn rat ovary (Beaumont & Mandl 1962), while in the pig half the peak number survive in the newborn (Black & Erickson 1968). The reduction of the number of oocytes which is already in progress at the time of oogenesis is completed, continues with advancing age (Jones & Krohn 1961). The factors responsible for this atresia are not yet entirely known.

Concluding remarks

It is quite certain by now that the germ-cell line in the mammal is a continuous one, from the cell that migrates into the gonad and multiplies, to that which enters meiosis and becomes the mature sex cell. The stages of differentiation of the germ cells are well defined and the time of DNA synthesis in them is known. However, the dynamics of their differentiation is as yet quite unexplored. The stimuli which are necessary to halt temporarily proliferation in the male germ cell in the embryo, and the stimulus which is necessary for the female germ cell to stop mitosis permanently and enter the long meiotic prophase are not yet known. Furthermore, the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

developmental difference which presumably exists between those animals in which oogenesis goes on during embryonic life and those in which it is delayed until the neonatal period has not yet been defined. In those species so far examined in which ovulation occurs on stimulation only, oogenesis takes place in the neonatal period, while in those (with one exception) where ovulation is spontaneous, oogenesis occurs within the foetal period. This might suggest possible correlations (a) between oogenesis taking place during embryonic life and cyclic release of gonadotrophin leading to spontaneous ovulation in the mature animal, and (b) between oogenesis in the neonatal period and non-cyclic release of gonadotrophins.

References (Peters)

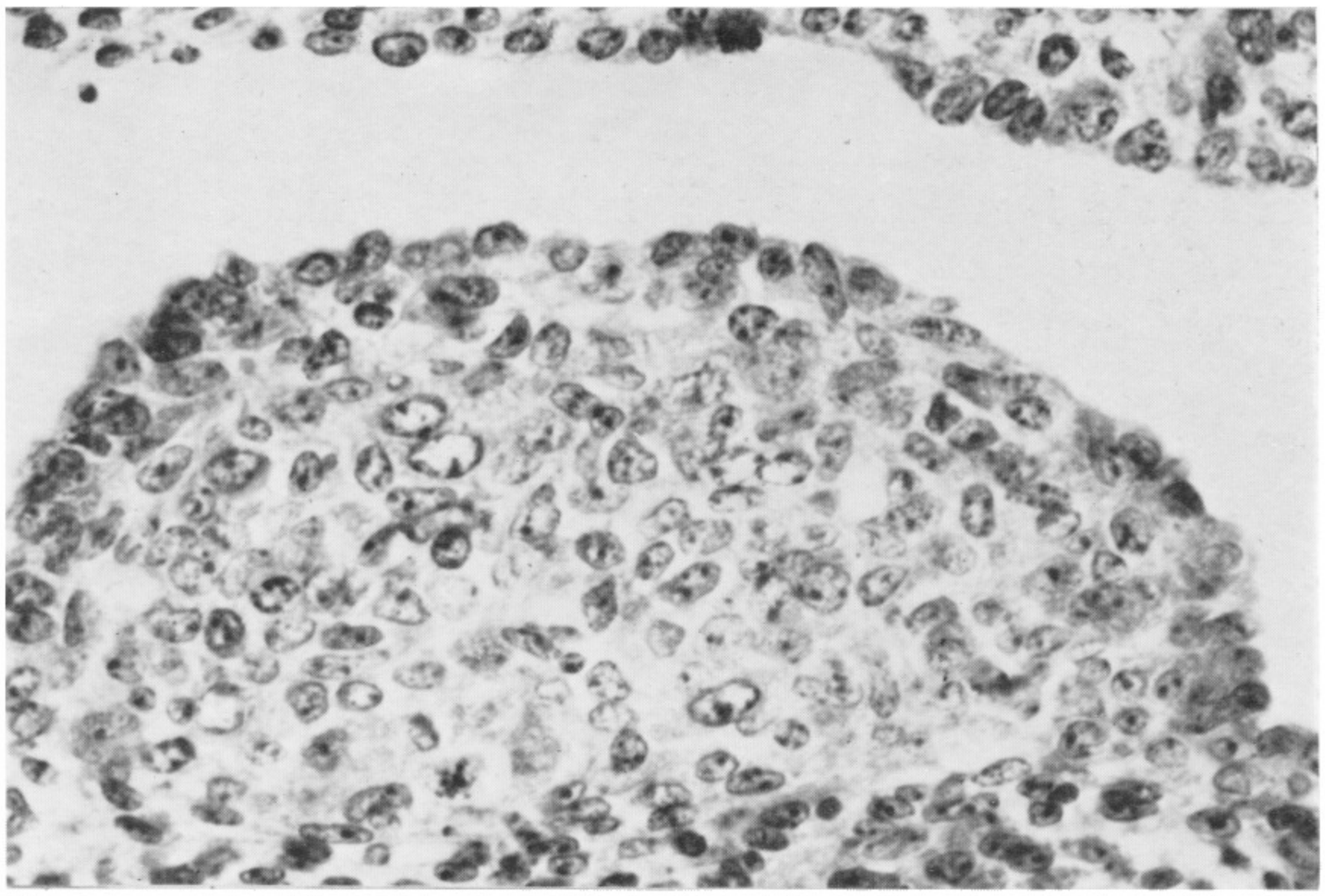
Baker, T. G. 1963 *Proc. Roy. Soc. Lond.* B **158**, 417.
Baker, T. G. 1966 *J. Anat.* **100**, 761.
Baker, T. G. & Franchi, L. L. 1967 *J. Cell Sci.* **2**, 213.
Beaumont, H. M. & Mandl, A. 1962 *Proc. Roy. Soc. Lond.* B **155**, 557.
Beaumont, H. M. & Mandl, A. 1963 *J. Embryol. exp. Morph.* **11**, 715.
Black, J. L. & Erickson, B. H. 1968 *Anat. Rec.* **161**, 45.
Blandau, R. J., White, B. J. & Rumery, R. E. 1963 *Fert. Steril.* **14**, 482.
Borum, K. 1961 *Expl Cell Res.* **24**, 495.
Borum, K. 1966 *Expl Cell Res.* **45**, 39.
Brambell, F. W. R. 1927 *Proc. Roy. Soc. Lond.* B **101**, 39.
Brambell, F. W. R. 1956 In *Marshall's physiology of reproduction*, vol. 1, p. 397 (ed. A. S. Parkes). London, New York and Toronto: Longmans, Green and Co.
Chiquoine, A. D. 1954 *Anat. Rec.* **118**, 135.
Clermont, Y. & Bustos-Obregon, E. 1968 *Am. J. Anat.* **122**, 237.
Clermont, Y. & Perey, B. 1957 *Am. J. Anat.* **100**, 241.
Courot, M. 1962 *Annls. Biol. anim. Biochim. Biophys.* **2**, 25.
Crone, M., Levy, E. & Peters, H. 1965 *Expl Cell Res.* **39**, 678.
Deanesley, R. 1970 in press.
Erickson, B. M. 1966 *J. Reprod. Fert.* **10**, 97.
Everett, N. B. 1943 *J. exp. Zool.* **92**, 49.
Fawcett, D. W. 1961 *Expl Cell Res.* (Suppl.) **8**, 174.
Fawcett, D. W., Susumo, I. & Slautterback, D. 1959 *J. biophys. biochem. Cytol.* **5**, 453.
Franchi, L. L. & Mandl, A. M. 1962 *Proc. Roy. Soc. Lond.* B **157**, 99.
Franchi, L. L. & Mandl, A. M. 1964 *J. Embryol. exp. Morph.* **12**, 289.
Franchi, L. L., Mandl, A. M. & Zuckerman, S. 1962 In *The ovary*, vol. 1, p. 1 (ed. S. Zuckerman, A. M. Mandl and P. Eckstein). London: Academic Press.
Gondos, B. & Zamboni, L. 1967 *J. Ultrastruct. Res.* **21**, 162.
Gondos, B. & Zamboni, L. 1969 *Fert. Steril.* **20**, 176.
Greenwald, G. S. & Peppler, R. D. 1968 *Anat. Rec.* **161**, 447.
Hargitt, G. T. 1926 *J. Morph.* **42**, 253.
Herlant, M. 1960 *Annls. Soc. roy. zool. Belg.* **91**, 1.
Hilscher, W. & Makosi, H. B. 1968 *Z. Zellforsch. mikrosk. Anat.* **86**, 327.
Huckins, C. 1963 *Anat. Rec.* **145**, 243.
Ioannou, J. M. 1964 *J. Embryol. exp. Morph.* **12**, 673.
Jones, E. & Krohn, P. 1961 *J. Endocr.* **21**, 469.
Kennelly, J. J. & Foote, R. H. 1966 *Am. J. Anat.* **118**, 573.
Kumar, A. 1968 *Proc. Roy. Soc. Lond.* **169**, 167.
Lima-de-Faria, A. & Borum, K. 1962 *J. Cell Biol.* **14**, 381.
Mauleon, P. 1961 *Compt. rend. 4^eme Congres Int. Reprod. Animale*, p. 348, La Haye.
McKay, D. G., Hertig, A. T., Adams, E. C. & Danziger, S. 1953 *Anat. Rec.* **117**, 201.
Mintz, B. 1959 *Anat. Micr. Morphol. exp.* **48**, (Suppl. 155).
Mintz, B. 1960 *J. cell. comp. physiol.* **56**, (Suppl. 1), 31.
Mintz, B. & Russell, E. S. 1957 *J. exp. Zool.* **134**, 207.
Nebel, B. R., Amarose, A. P. & Hackett, E. M. 1961 *Science, N.Y.* **134**, 832.
Novi, A. M. & Saba, P. 1968 *Z. Zellforsch. mikrosk. Anat.* **86**, 313.
Peters, H. & Crone, M. 1967 *Archs. Anat. microsc. Morph. exp.* **56**, (Suppl. 3–4), 160.
Peters, H. & Levy, E. 1966 *J. Reprod. Fert.* **11**, 227.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

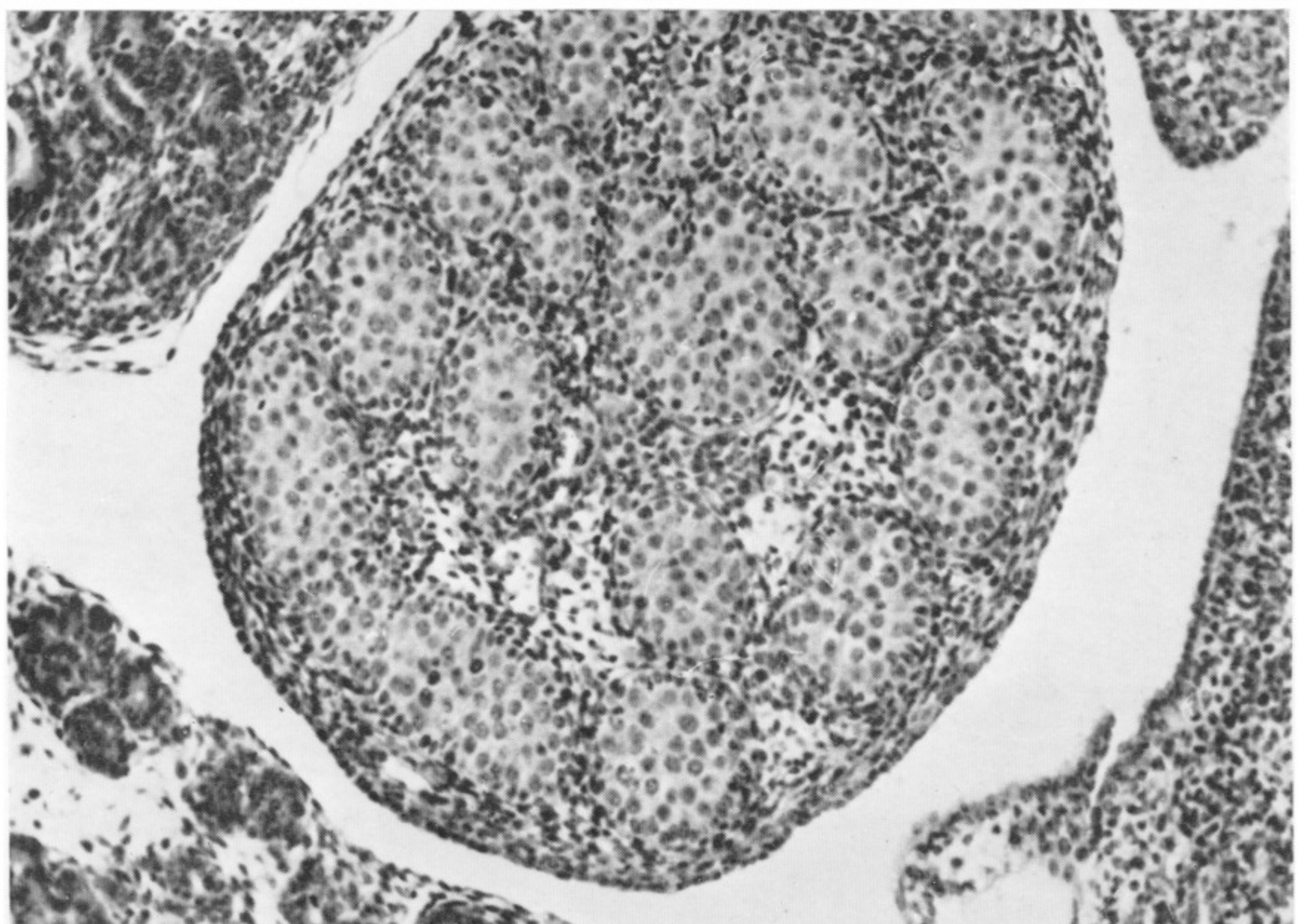
Peters, H., Levy, E. & Crone, M. 1962 *Nature, Lond.* **195**, 915.
Peters, H., Levy, E. & Crone, M. 1965 *J. exp. Zool.* **158**, 169.
Petter-Rousseaux, A. & Boulière, F. 1965 *Folia primat.* **3**, 241.
Roosen-Runge, E. C. & Leik, J. 1968 *Am. J. Anat.* **122**, 275.
Rudkin, G. T. & Griech, H. A. 1962 *J. Cell. Biol.* **12**, 169.
Sapsford, C. S. 1962*a* *Aust. J. Zool.* **10**, 178.
Sapsford, C. S. 1962*b* *Aust. J. agric. Res.* **13**, 487.
Simkins, C. S. 1923 *Acta zool., Stockh.* **4**, 241.
Teplitz, R. L. & Ohno, S. 1963 *Expl Cell Res.* **31**, 183.
Van Wagenen, G. & Simpson, M. E. 1965 *Embryology of the ovary and testis.* New Haven and London: Yale University Press.
Weakley, B. S. 1967 *J. Anat.* **101**, 435.
Winiwarter, H. von 1901 *Archs. Bid., Paris* **17**, 33.
Winiwarter, H. von & Saimont, G. 1909 *Archs. Biol., Paris* **24**, 165.
Witschi, E. 1948 *Contr. Embryol. Carnegie Inst.* **37**, 80.
Witschi, E. 1962 In *The ovary*, Int. Acad. of Pathol. Monograph No. 3, Baltimore: Williams and Wilkins.
Zamboni, L. & Gondos, B. 1968 *J. Cell. Biol.* **36**, 276.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

1



2



3

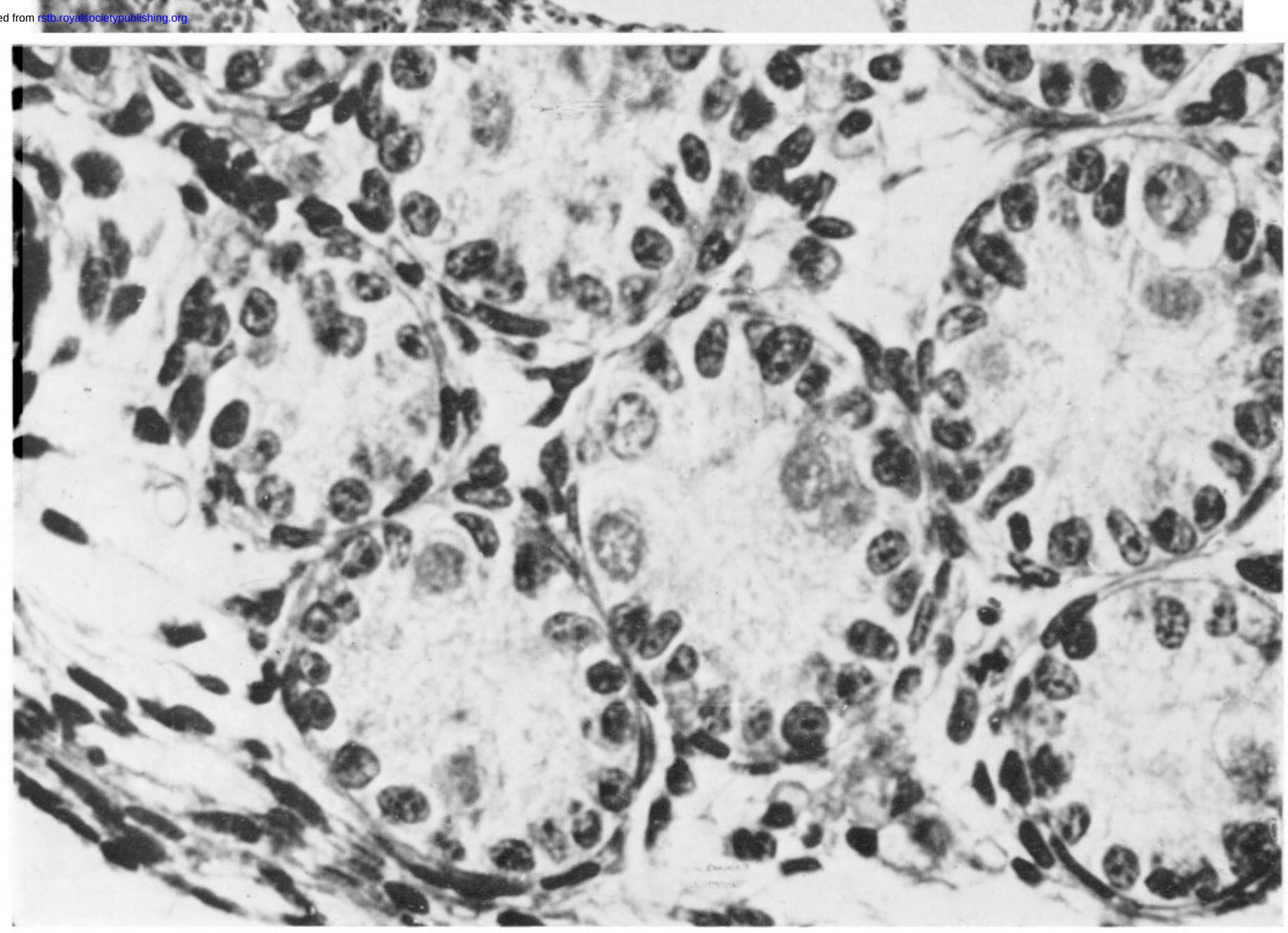
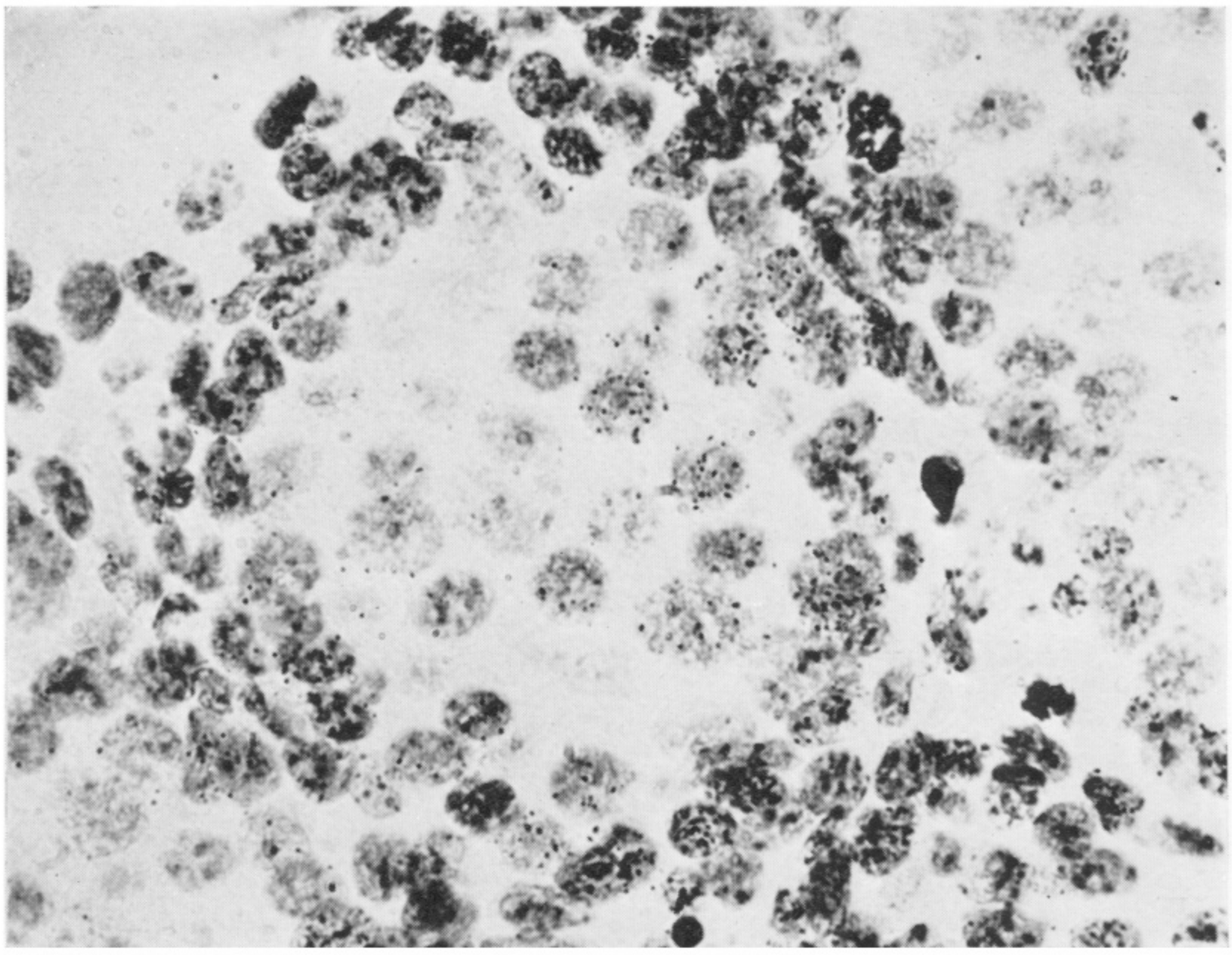


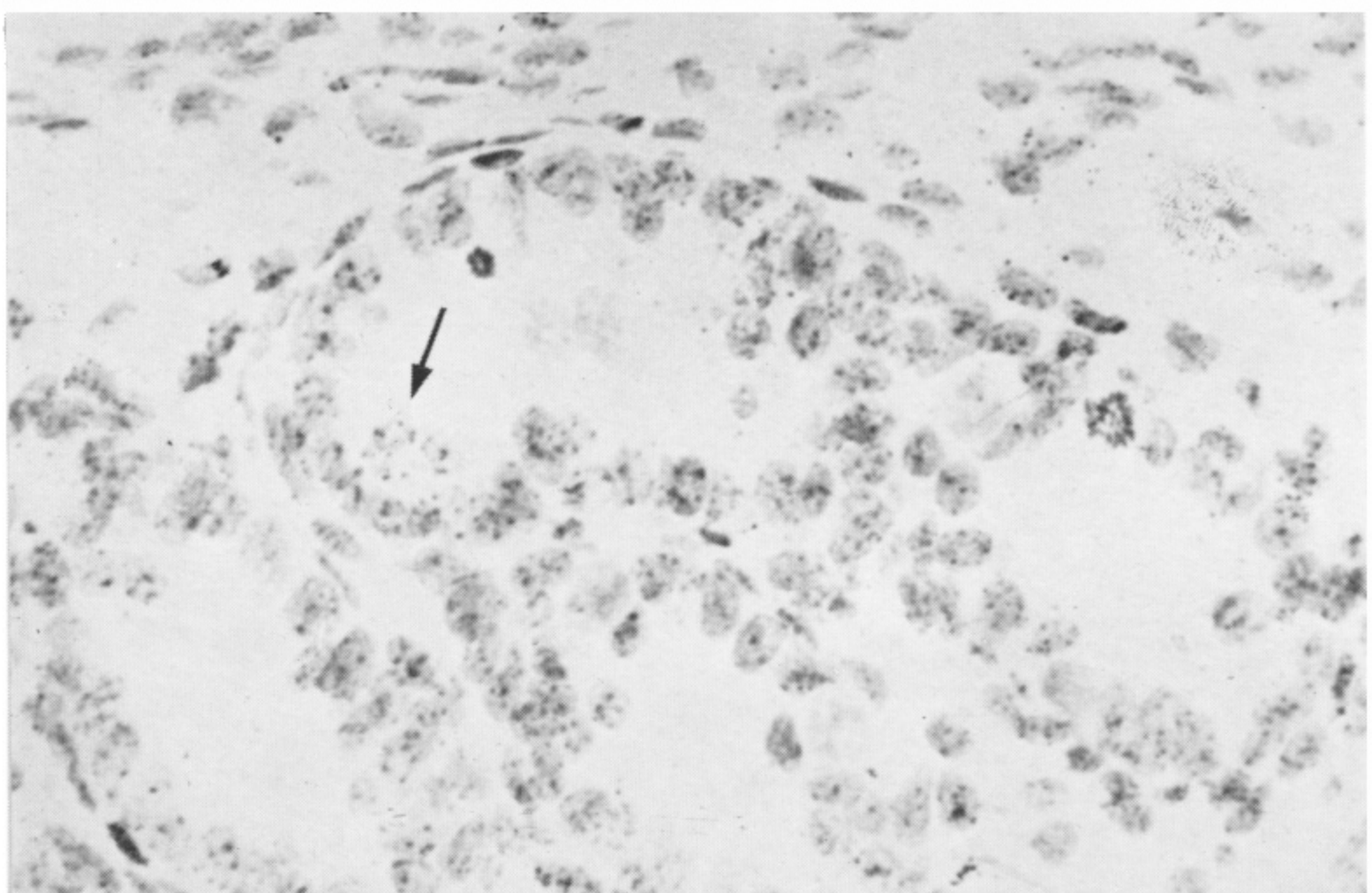
FIGURE 1. Undifferentiated gonad in a 12-day-old mouse embryo (magn. × 510).

FIGURE 2. Testis of a 16-day-old mouse embryo. Gonocytes lie in the centre of the seminiferous cord (magn. × 153).

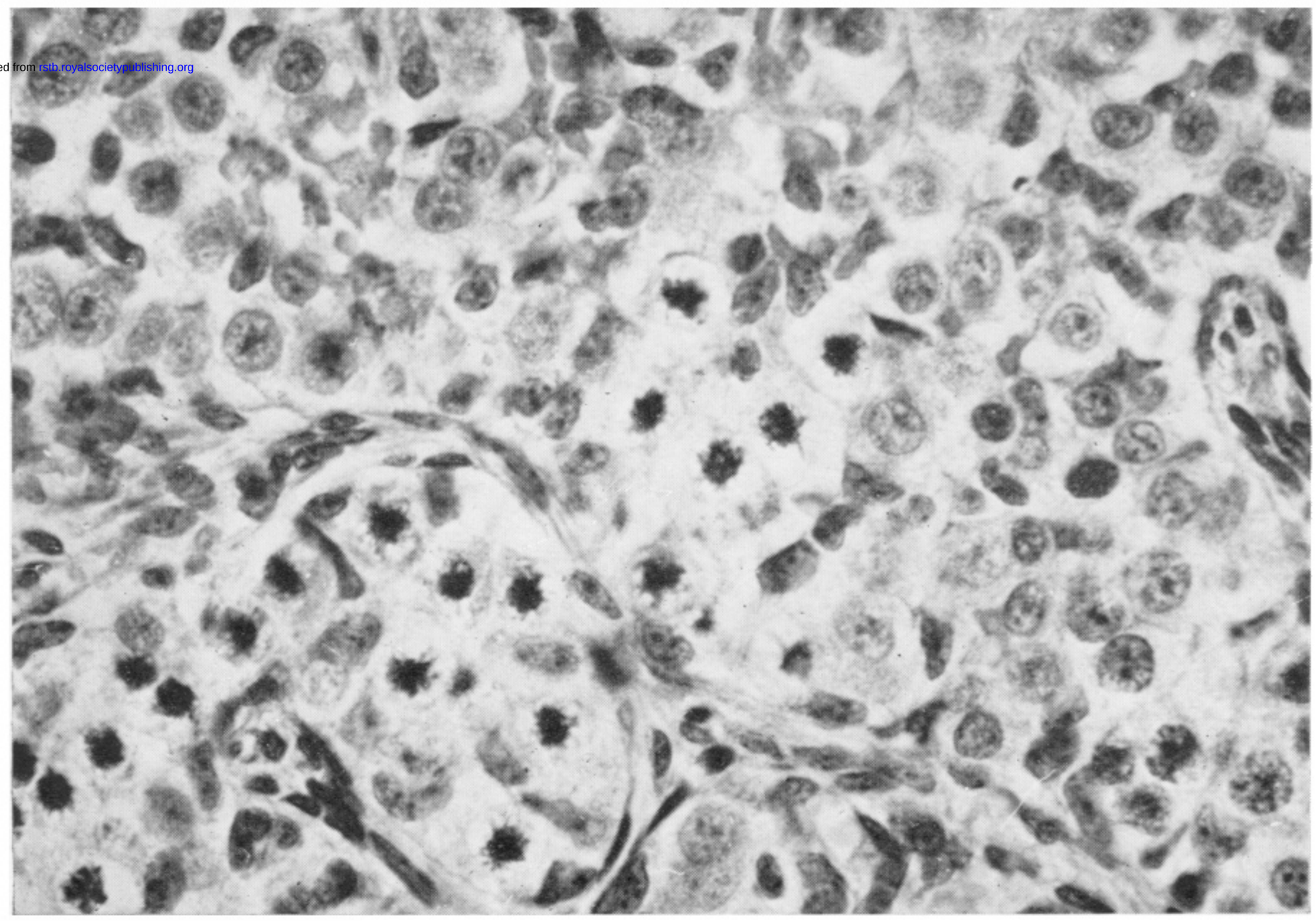
FIGURE 3. Testis of a newborn mouse. Some gonocytes are seen lying close to the basement membrane in the seminiferous cords (magn. × 510).



4



5



8

FIGURE 4. Labelled gonocytes in the testis of a 15-day-old embryo that had received ^{3}HTdR 2 days earlier (magn. × 540).

FIGURE 5. Labelled gonocyte (↑) seen in the autoradiograph of the testis of a 3-day-old mouse that had received ^{3}HTdR on the 13th day of embryonic life (magn. × 210).

FIGURE 8. Ovary of a 4-day-old rabbit with groups of oogonia and groups of oocytes in early stages of meiotic prophase (magn. × 510).