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How Does the Y Chromosome Affect Gonadal Differentiation? [and Discussion]

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How does the Y chromosome affect gonadal differentiation?

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[Plate 13]

The problem of sex differentiation in mammals is based on three sets of data.

First, according to the embryological evidence, the gonads appear at first morphologically identical in both sexes and subsequently develop into ovaries or testes according to the sex chromosome constitution (van Wagenen & Simpson 1965).

The second set of data is based on the discovery by Jost that the embryonic testis plays a decisive role in the development of the male anatomical characteristics. By contrast, the embryonic ovary appears not to be necessary for the development of the female anatomical features (Jost 1965).

Lastly, the results of cytogenetics have made it clear that the Y chromosome is male determining in man (Ford 1963) and the mouse (Cattanach 1961; Morris 1968) and it is probably reasonable to make the tentative assumption that the male-determining capacity applies to the Y chromosomes of mammals in general.

The problem of chromosomal sex differentiation therefore resolves itself into the question: 'How does the mammalian Y chromosome turn the apparently indifferent gonad into a testis?'

Attempts to answer this question in genetic terms may be divided into three categories.

The simplest hypothesis assumes a single gene situated on the Y chromosome to be responsible for testicular development. Although this idea is sometimes expressed in the recent literature on human cytogenetics, it is almost certainly an over-simplification, since it takes no account of the apparent bipotentiality of the gonads, the findings on sex reversal in lower vertebrates (Witschi 1956) or the different abnormalities of sexual development in man (Federman 1967). To accommodate these facts and also to bring the subject of mammalian sex determination into line with classical theories based on *Drosophila* (Bridges 1925) and the moth, *Lymantria* (Goldschmidt 1934), large numbers of male- and female-determining genes have been postulated in both sexes, with the genes for one sex normally outweighing the genes for the other sex (Ford 1963). Male-determining genes would be expected to occur on the mammalian Y chromosome as well as on other chromosomes. An intermediate position is exemplified by the suggestion made by Hamerton (1968) that both sexes carry a gene each for testicular and ovarian development and that the Y chromosome carries a controlling element which allows the testicular gene to function.

However, the evidence for the existence of sex-determining genes has not, so far, been conclusive and the question may be raised whether sex differentiation may be the result of differential growth processes in the embryo (Mittwoch 1967, 1969). This would mean that the basic difference between maleness and femaleness is a quantitative one and that a threshold mechanism channels development in one of two directions. The mechanism by which a quantitative difference gives rise to two qualitatively different end products appears to be well

documented. Grüneberg (1952) has called the phenomenon 'quasi-continuous variation' and Wright, who first described it in 1934, calls it a 'threshold dichotomy' (Wright 1968). As an example of threshold dichotomy, Wright (1968) cites the formation of two sexes in species which do not have sex chromosomes. I suggest that the same process may apply in organisms in which sex chromosomes are present and that in these it is the sex chromosomes which bring about the quantitative variation as well as the threshold mechanism.

Recent evidence suggests that, in plants as well as in mammalian cells, different chromosome constitutions may influence the length of the mitotic cycle and, therefore, affect the rate of cell proliferation. The general rule appears to be that the more DNA present per nucleus, the longer the time taken for DNA synthesis, resulting in a slowing down of the mitotic rate (Van't Hof 1965; Lennartz, Schümmelfeder & Maurer 1966; Ayonoadu & Rees 1968). On the other hand, it may be possible that in special circumstances the addition of an extra chromosome may increase the number of mitoses (Darlington & Thomas 1941).

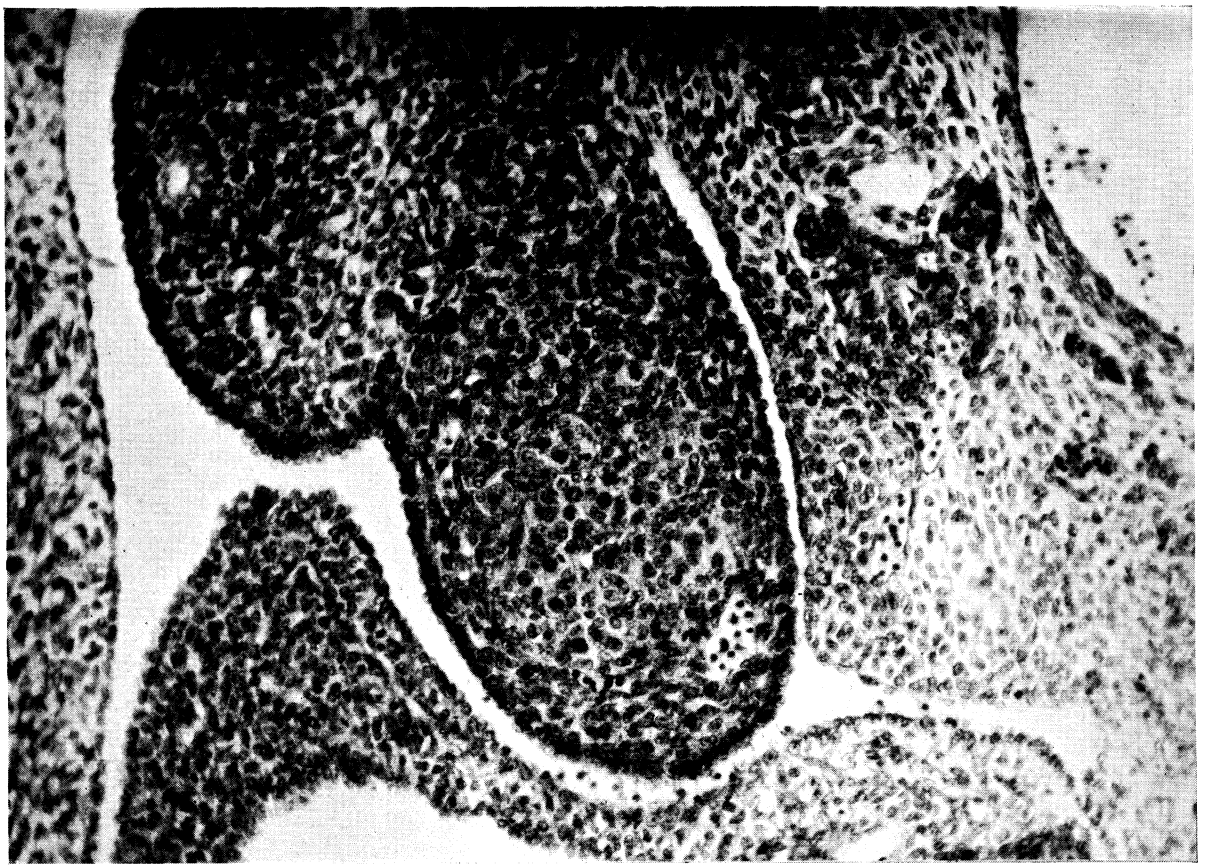
Owing to the difference in size between the X and the Y chromosome in man, the amount of DNA per cell in females is nearly 2 % more than in males (Chicago Conference 1966). If one assumes that the time taken for DNA synthesis is proportional to the amount of DNA, DNA synthesis would be a slightly slower process in females and, other things being equal, the rate of cell proliferation would be slower in females than in males. This would be in accordance with the smaller size of women, but it could not account for sex differentiation. For this, a positive role of the Y chromosome is required. I suggest that at a given stage in the development of the embryo, the Y chromosome may boost the already slightly increased growth rate of male embryos, possibly by increasing the number of mitoses in the developing gonads. This might cause the gonads to develop into testes, which will then produce hormones and these, in turn, will control the subsequent male development of the embryo.

Do differentiating testes, in fact, grow faster than ovaries? It seemed that this question might be answered most easily in a small mammal with a large litter, containing male and female embryos of the same ages, and in which the sex chromosomes of the male and female could be distinguished. The rat appeared to be a likely animal to fulfil these requirements.

The experiments were carried out in conjunction with Dr Felix Beck and Dr Joy Delhanty. Male and female albino rats were put together overnight, vaginal smears were made the following morning and litters of embryos were dissected out at various ages. The head or forelimbs from each embryo were set up for cell culturing, the yolk sac was fixed in 95 % ethanol for Feulgen staining and sex chromatin analysis and the abdomen was serially sectioned at 10 μm. The volume of a gonad was estimated by measuring the two largest diameters of every sixth section with an eye piece micrometer. On the assumption that the areas of the cross-sections are ellipses (figure 1, plate 13) the volume was calculated as $30\pi\ (a_1\ b_1 + 2a_2\ b_2 + 2a_3\ b_3 \ldots + a_n\ b_n)$, where a and b are half diameters.

The karyotype of the rat (*Rattus norvegicus*) has been described many times (see Benirschke & Hsu 1967). The diploid chromosome number is 42 and the X chromosome is a medium-sized acrocentric, and the Y a small acrocentric chromosome (figure 2). The sex of an embryo could also be determined from the presence or absence of Barr bodies in cultured fibroblast cells (figure 3, plate 13) or in yolk-sac cells (figure 4, plate 13). For the latter purpose, squash preparations from bulk stained material were made.

The gonadal volumes from a litter of embryos aged $14\frac{1}{2}$ days are shown in table 1. At this age, male and female gonads can just be distinguished on histological grounds (Hennenberg



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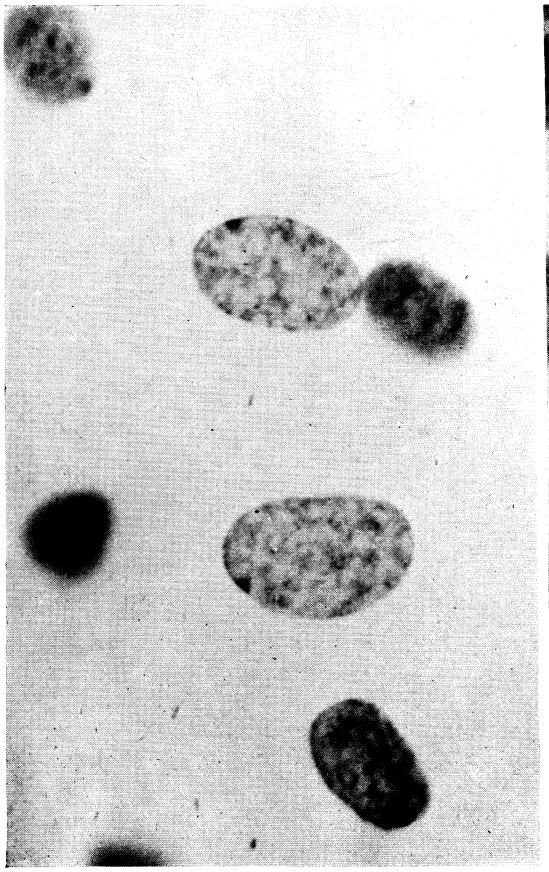

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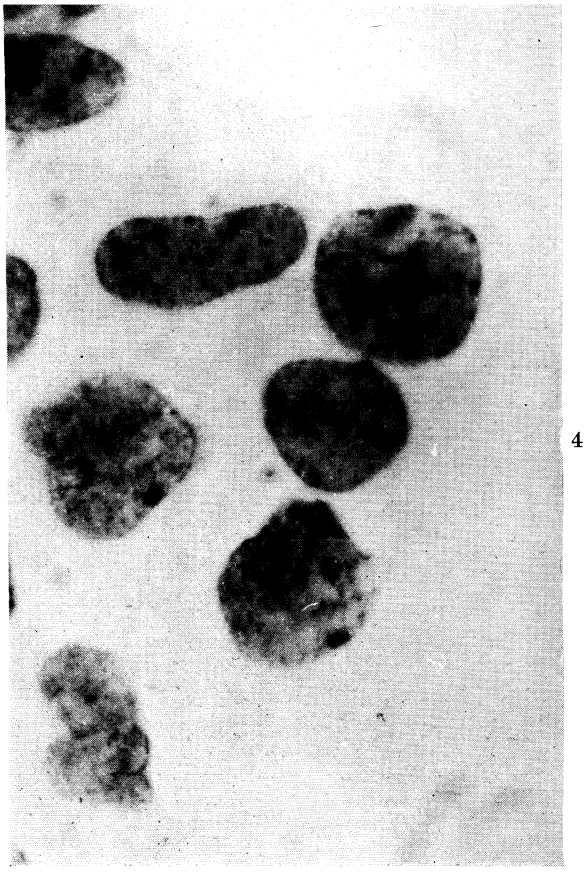

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FIGURE 1. 14½-day-old male embryo, cross-section through gonad. (Magn. × 250 approx.)

FIGURE 3. Barr bodies in nuclei of fibroblast. (Magn. × 1500 approx.)

FIGURE 4. Barr bodies in nuclei of yolk sac. (Magn. × 1500 approx.) (*Facing p.* 114)

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1937). One gonad from each embryo was measured. It will be seen that, on an average, the male gonads were 40 % larger than the female ones.

When these results had been obtained, my attention was drawn to a thesis published by Lindh (1961) in the University of Lund. The author had measured male and female gonads in embryos of rats and golden hamsters and found the testes to be larger in both species. The two sets of independent observations are therefore mutually confirmative.

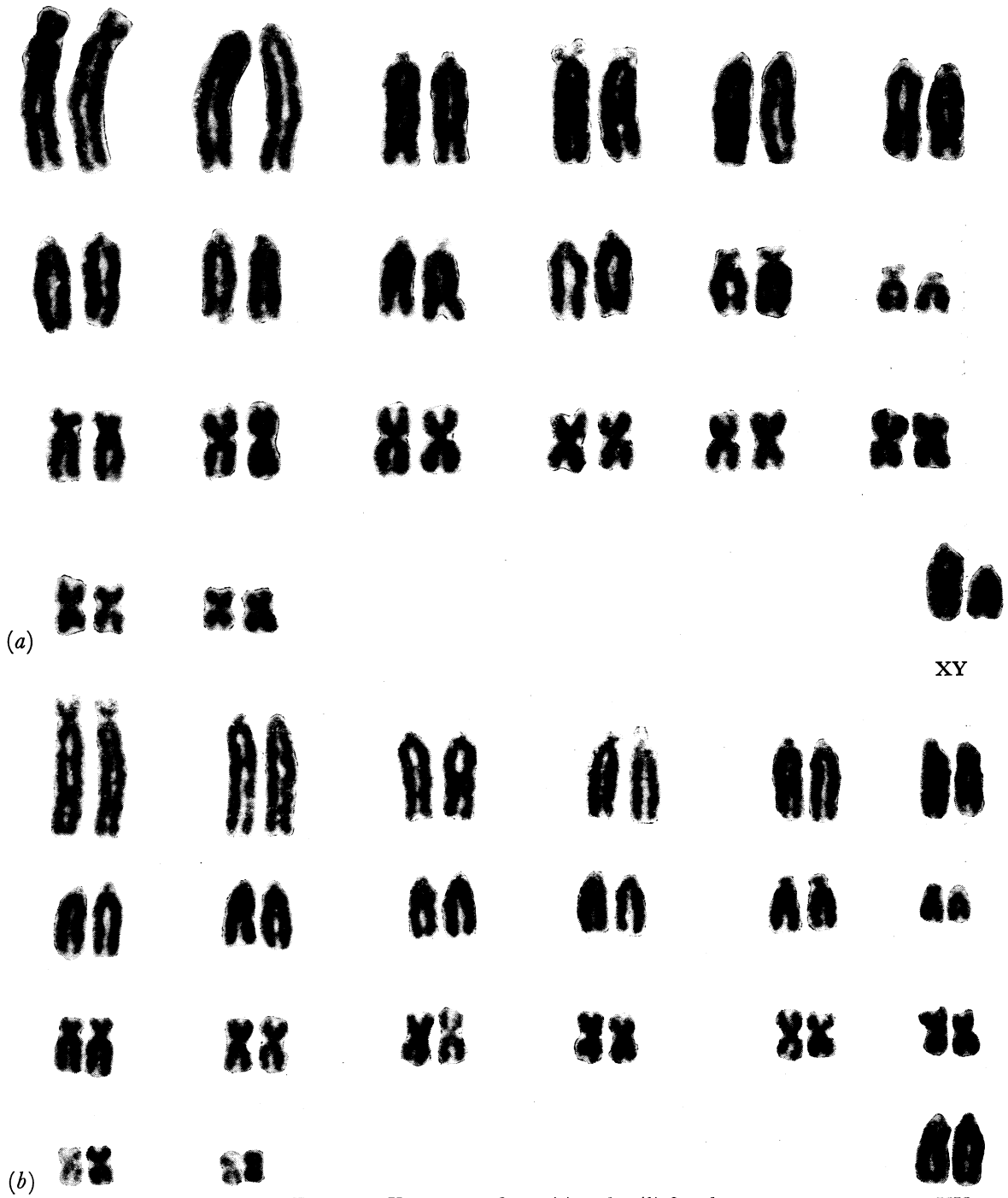

FIGURE 2. Karyotype of rat: (*a*) male; (*b*) female.

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TABLE 1. GONADAL VOLUMES IN RAT EMBRYOS ($14\frac{1}{2}$ days)

	♂	♀
	($\times 10^5$ μm^3)	
	310	171
	286	209
	264	155
		160
		240
		242
		248
mean	287	204
t	3.22	
P	0.02 – 0.01	

It is not yet known what causes the increased size of the testis. However, an effect of the Y chromosome in increasing the number of cell divisions might fit in with two lines of evidence:

(1) In lower vertebrates, sex reversal can sometimes be brought about by changes in temperature (Witschi 1942). This suggests that the sex difference may be affected by alterations in rates of growth.

(2) Experiments by Hadorn (1968) have shown that cells of the imaginal disks in *Drosophila* larvae are determined to form definite organs in the adult but that the determination can be changed, and different organs formed, by subjecting the cells to conditions of active cell proliferation.

One might speculate therefore that the mammalian gonad is destined to form an ovary but that, if a Y chromosome is present, it will cause an increase of cell division in the gonad, which will switch development into that of a testis.

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Discussion on papers by A. K. Tarkowski, p. 107 *and U. Mittwoch, p.* 113

J. L. Hamerton (*Guy's Hospital Medical School, London*): Short and I have been analysing the genetically determined intersexual state in the goat, where a gene determines medullary development when homozygous in females and so leads to the development of a testis in these females. With one exception, which was a chimaera, there is no question but that the animals were XX. In no case could we find germ cells in any adults, and we therefore set up breeding experiments to produce intersex foetuses to find out if the germ cells never reached the testis or degenerated later in development. We found the germ cells arrived in the testis (let us stress that these are XX germ cells in an XX soma) and they persisted up to 120/130 days of gestation out of a total period of 150 days. By birth they had completely degenerated.

We do not know what happens during the critical 20 days. In one testis the germ cells appeared to be abnormal and degenerating at this time, although in most cases these cells were perfectly normal. In one animal which was a true hermaphrodite with true testicular and ovarian tissue we found normal germ cells in the testis and follicles containing dictyate oocytes in the ovary at 120 days.

R. G. Edwards: There are two types of somatic cells in the chimaeras produced by Tarkowski, and various combinations of germ cells and soma are possible. Is it known what happens with various combinations of germ cells and specific somatic cells?

A. K. Tarkowski: Several non-gonadal tissues as well as the germinal tissue of experimental mouse chimaeras have been tested and found chimaeric. Unfortunately, corresponding data on the composition of the somatic tissue of the gonads are not available up to the present. It is very likely that the gonads of sex chromosome chimaeras contain originally germ cells as well as somatic cells of two genetic sexes, in proportions and spatial configurations varying from one gonad to another. Although this complicates the picture, the gonad of a sex chromosome chimaera appears to be a very convenient experimental system for studying the interactions between the germinal and the somatic tissue. The best experimental system for studying this type of interaction is that described in birds by Dr Haffen, who succeeded in placing primordial germ cells of one genetic sex into the sterile gonad of the other sex (Haffen 1968 *6th International Congress of Embryology, Paris*). It would be difficult, however, to perform such an experiment in mammals.

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A. Jost: Sex differentiation of the gonad can be understood without postulating two different kinds of inductors or a competitive system between the cortex and the medulla. The Y chromosome could induce a change in a system which otherwise would develop as an ovary. I am therefore prepared to accept some of Dr Mittwoch's suggestions, although an unspecific effect on mitosis and cell division probably would not suffice to explain the complex process of testicular organogenesis.

U. Mittwoch (*University College, London*): I agree that the situation is likely to be more complicated than I suggested. Nevertheless, the influence of cell division on differentiation is, I think, worth considering. The experiments by Hadorn, which I mentioned very briefly, may be relevant to this point. Hadorn and his colleagues found that embryonic disk cells in *Drosophila* larvae are destined to form definite organs in the adult flies. For instance, cells of the male genital disk would develop into sex organs following a number of transplant generations. However, after a time, some of the cells would develop into parts of the head and legs. This change in differentiation was found to be correlated with the rate of cell proliferation. These results suggest that the rate of cell division may be implicated in the process of differentiation.

C. E. Ford: Kahn, Edwards and I studied the role of somatic cells in two cases of human XO/XY mosaics. Both of them had been raised as boys, and both had ambiguous external genitalia. At laparotomy at 3 and 13 years of age respectively biopsy material was taken for culture from a testis and from an ovarian streak from both patients. Testes from one of the boys produced 30% XY cells and 70% XO, whereas the ovarian streak gave 10% XY, 90% XO. In the second boy, the testis contained 20% XY, 80% XO, and the ovarian streak gave 10% XY, 90% XO. This evidence is only slight, but indicates that the Y chromosome may have a threshold effect upon gonadal differentiation depending upon the proportion of cells that contain it in the gonadal ridge. Human cases of pure gonadal dysgenesis can be either XX or XY and have indistinguishable phenotypes. Could Dr Mittwoch comment on this?

U. Mittwoch: Undetected mosaicism is one explanation. However, if the effect of the Y chromosome is to increase the rate of growth, this would be a very non-specific effect and one could imagine many causes, both genetic and environmental, which might either simulate or counteract it.

A. McLaren: I have the impression that there is an evolutionary trend towards a more determinate, less labile sex-determination mechanism. In certain invertebrate groups the environment can determine the sex of an individual. In fish, germ cells of the same genetic sex can develop as male or female according to the age of the individual. In Amphibia, female germ cells in a male differentiate into spermatozoa, and male germ cells in a female differentiate as eggs. However, in mammals and birds this is no longer so. Whether a mammalian germ cell differentiates in the male or the female direction seems to depend not at all on its environment, but only on its own chromosomal constitution. What the selective advantage might be of the mammalian as opposed to the amphibian system is not obvious.

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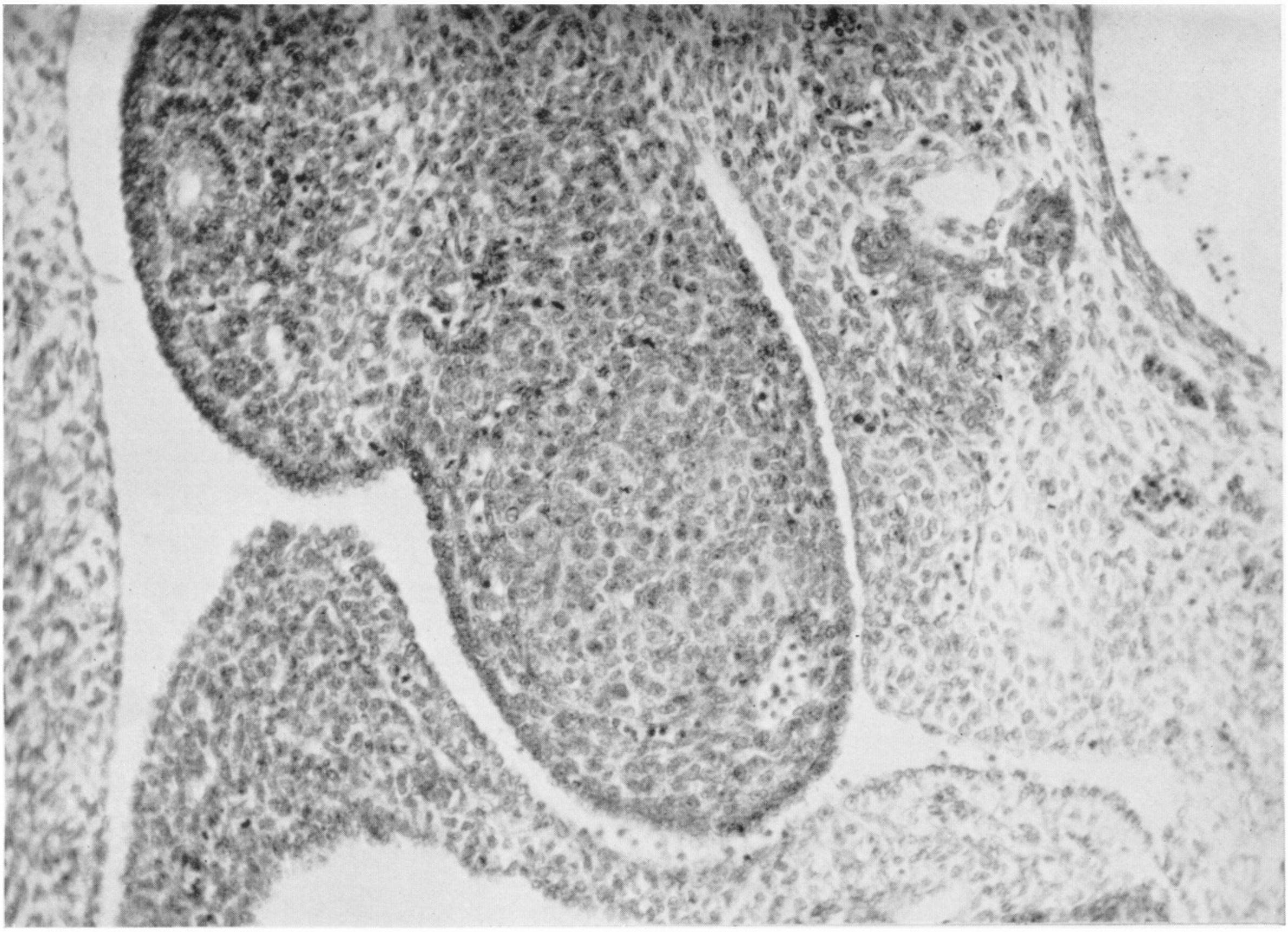

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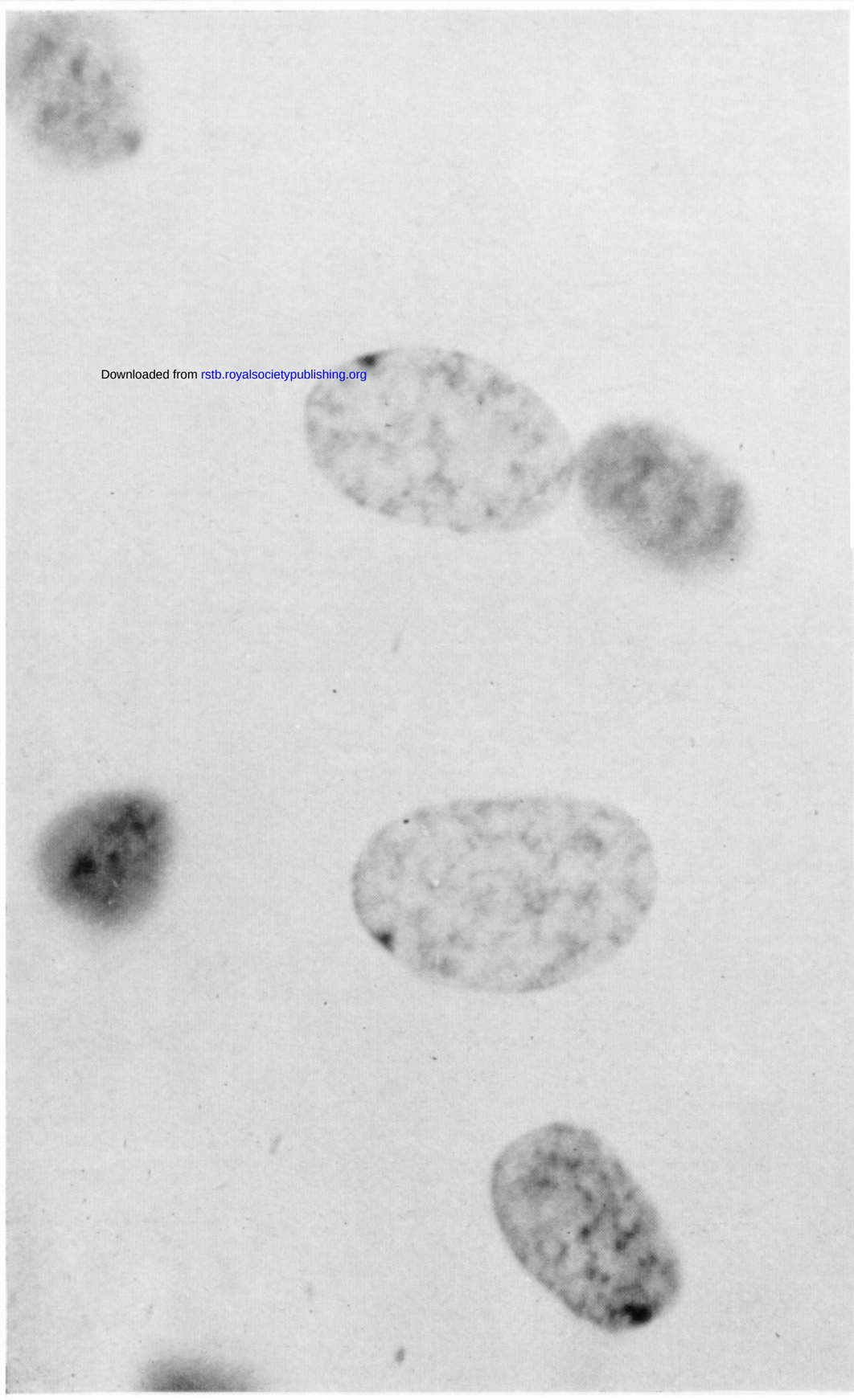

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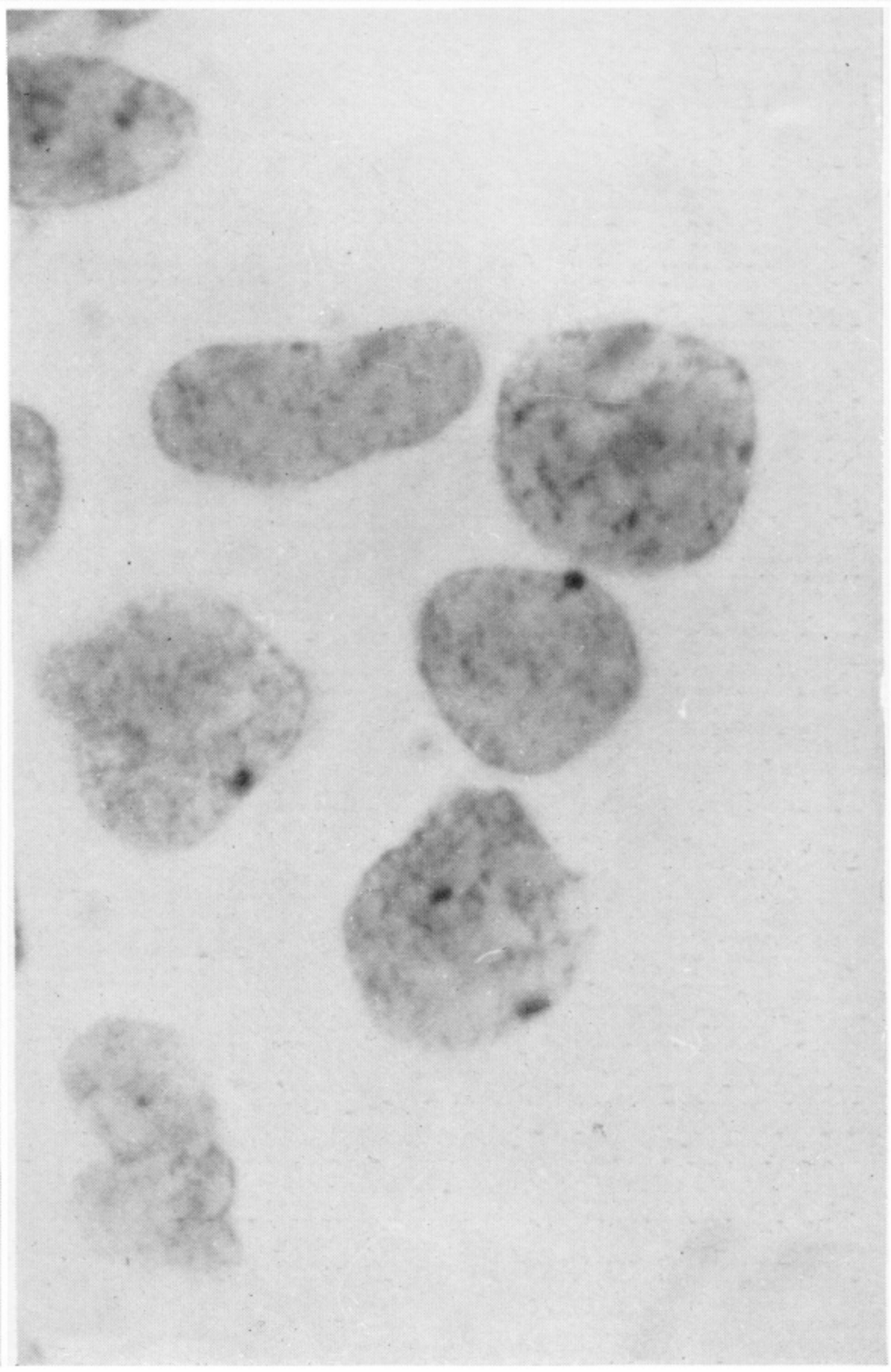

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