Sexual Dimorphism of Rat Cells in vitro

Abstract. Large peripheral chromocenters occurred in about 10 percent of the nuclei of female rat cells grown in vitro but occurred on the average in less than 1 percent of the nuclei of male cells. Similar chromocenters occurred in hamster and mouse nuclei in vitro, but no clear-cut sexual difference was demonstrable.

A distinct sexual dimorphism has been established for the interphrase nuclei of many mammalian species, especially in the orders Primate and Carnivora (*1*). This dimorphism depends on the presence of the sex chromation body of Barr in the nuclei of female cells. Study of the metabolic and genetic aspects of Barr chromatin might be facilitated if the chromatin were found to be present in a small laboratory animal such as the rat, mouse, or hamster. Moore and Barr (*2*) were unable to detect a nuclear sex difference in these animals. Subsequently, however, sex chromatin was reported in motor neurons (*3*), liver cells (*4*), and ameloblasts (*5*) of female rats, although in the first two of these reports the relative incidence is not specified. In addition, it has been reported that sex chromatin occurs in the neurons of female golden hamsters (*6*); again, the incidence is not specified.

Hinrichsen and Gothe (*7*) have recently published a detailed study of the chromatin patterns in the rat and mouse. They report that nearly half the nuclei of Purkinje cells of female rats have a solitary peripheral chromocenter. While they did not find many peripheral chromocenters in mouse nuclei, they do report a sex difference based on the size and number of nucleolar chromocenters. Although exact parallelism between the chromatin patterns of cells in vitro and in vivo remains to be demonstrated, it has been noted that the sex chromatin body may be more readily identifiable in tissue cultures, and in at least one case (*8*) the sex chromatin body has been demonstrated in culture when it could not be identified in tissue section.

With the thought that a comparable situation might obtain with respect to the more complex chromatin patterns in rodents, we employed two methods of cell culture, using heart, spleen, and kidney from the Sprague-Dawley strain of *Rattus norvegicus* (*9*). Three rats of each sex were sacrificed. Tissues were trypsinized, and aliquots were plated and incubated on cover slips in petri dishes, by a method described elsewhere (*10*). Small fragments were also explanted in plasma clots on cover slips by the method of Southam and Goettler (*11*). Cultures were incubated for periods ranging from 6 to 33 days, after which the cover slips were treated in accordance with the thionin staining procedure of Klinger and Ludwig (*12*).

Many nuclei showed a solitary peripheral chromatin mass which varied from about 0.5 to 1.0 μ in width and from about 1.0 to 1.8 μ in length (average, about 0.8 by 1.4 μ). As shown in Table 1, the incidence in female tissues varied from 1 to 22.1 percent and in male tissues, from 0 to 2.8 percent; all percentages are based on counts of at least 500 nuclei. Corresponding averages were 11.20 and 0.89 percent. There was no correlation with the tissue of origin or with the age of the culture. In general, cells from kidney tissue were epitheloid, while cells from heart and spleen were fibroblast-like. There did not appear to be any consistent difference in size or morphology between peripheral chromatin bodies in male and female cells.

Table 1. Percentages of peripheral chromocenters in 500 nuclei (S, spleen; H, heart; K, kidney).

Culture No.	Percentage	Tissue	Days cultured
	Female, trypsinized cells		
1	11.2	S	14
2	13.0	S	14
3	10.0	S	7
4	17.4	H	14
5	10.7	S	14
6	19.6	K	14
7	9.4	K	33
8	10.4	H	14
9	10.4	H	14
10	8.5	K	14
11	17.0	K	16
12	10.2	S	16
13	6.8	S	14
	Female, fragments explanted in plasma		
14	3.6	K	9
15	22.1	K	6
16	5.3	K	14
17	23.0	H	6
18	15.4	S	9
19	1.0	K	9
20	4.6	H	9
21	14.4	S	9
22	10.4	H	9
23	2.7	K	14
	Male, trypsinized cells		
1	0.3	S	7
2	1.0	S	17
3	1.2	S	7
4	2.8	S	7
	Male, fragment explants		
5	0	K	9
6	0	K	9
7	0.8	S	9
8	0.8	K	9
9	0.8	H	9
10	1.2	H	9

Fragment explants from heart, spleen, and kidney were also made from one individual of each sex of the golden hamster (*Cricetus auratus*) and from two individuals of each sex of the mouse (*Mus musculus*). No clear-cut sexual dimorphism was observed in these cultures. The hamster nuclei frequently showed solitary peripheral chromatin bodies. In seven female cultures the incidences were 10, 34, 19, 30, 36, 64, and 12 percent, respectively. In five male cultures the corresponding percentages were 4, 5, 11, 8, and 21. (These figures are based on counts of 100 nuclei.)

Mouse nuclei in vitro showed multiple chromocenters, the exact number often being difficult to determine since the chromocenters frequently vary in size down to the limits of visibility. However, a single nucleus may contain 10 to 20 large chromocenters (comparable in size to the sex chromatin body) and rarely lacks at least one or two. Such chromocenters often occurred at the nuclear membrane in both sexes. There was no apparent sex difference with respect to incidence or morphology. However, the morphologic variants often strikingly resembled those which have been described for sex chromatin in cultured human cells (*10*, *13*).

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References and Notes

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29 October 1959