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Human Testicular Cultures: I. Chromosome Analyses (38122)

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(Introduced by S. G. Bradley)

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It has been reported that hypodiploid cultures derived from carcinomas of the cervix can maintain growth *in vitro* for a period of 12–33 mo (1). Since hypodiploid chromosome constitution such as monosomic complement greatly facilitates genetic experiments (2), we wish to report an observation of a high frequency of hypodiploid cells in nongerminal human testicular cultures. The term 'hypodiploid' refers to chromosome number of 45 or less, regardless of the chromosome material present (1).

Materials and Methods. The testicular tissues used in this study were derived from 6 patients, one treated for cancer of the prostate (Patient 14C, aged 77), 2 treated for hydrocele (Patient 8C, aged 27; Patient 11C, aged 37), 2 had undescended testes (Patient 12C, aged 18; Patient 19C, aged 23) and the other had testicular feminization syndrome (Patient 18C, aged 12). The procedure for cultivating the testicular tissues is briefly described as follows: A few hours after the testicular biopsy, the tissue was cut into 1–2 mm square pieces in minimum essential medium with Hanks' salts (MEM) with antibiotics (penicillin 100 units/ml, streptomycin 100 μg/ml and amphotericin B 2.5 μg/ml). The small squares were washed several times in the antibiotic medium. About 6–8 pieces of tissue were then transferred to a 25 cm^2 Falcon flask with only a drop of medium (MEM supplemented with nonessential amino acids, 4m*M* glutamine, 10% fetal calf serum and antibiotics) surrounding each piece. The flasks were gassed with 5% CO_2 in air, screw capped and incubated at 37°. After 18–24 hr, 5 ml of fresh medium was added to the flasks. The flasks were gassed and incubated. The medium was then changed every three days, or as needed as indicated by the change of pH in the culture fluid.

Results. Three to five days after the cultures were initiated, nongerminal cells were observed growing from the explanted tissues. Many cells were seen surrounding the tubules (Fig. 1). Oftentimes, in the primary cultures, light microscopy and electron microscopy indicated the presence of two cell types, one epithelial-like and the other fibroblast-like. Cytochemically, the epithelial-like cells were shown to contain lipids (stained with Sudan IV, Fig. 2a) and glycogen (indicated by Periodic acid-Schiff reaction). Electron microscopic study showed that the epithelial-like cells had the characteristics of Sertoli cells (3) (Fig. 2b). Detailed results of this aspect of the study will be the subject of another report.

After the third passage, almost all the cells in the cultures appeared fibroblast-like. For the chromosome analyses in the beginning of the study, preparations were made from Patient 8C 2 mo after culture initiation (5th passage) and Patient 14C after one and one-half months (4th passage) using conventional procedures: Cultures were treated with deacetylmethyl colchicine (colcemid) at a concentration of 0.4 μg/ml of medium for 5 hr before harvesting. The cell suspension was centrifuged at 1,000 rpm and the cell button was suspended in 0.075 *M* KCl. The cells remained in the hypotonic solution for 8 min, were centrifuged and fixed in 3:1 methanol: acetic acid. Slides were flame-dried and then stained in Giemsa for 10 min. In Patient 8C,

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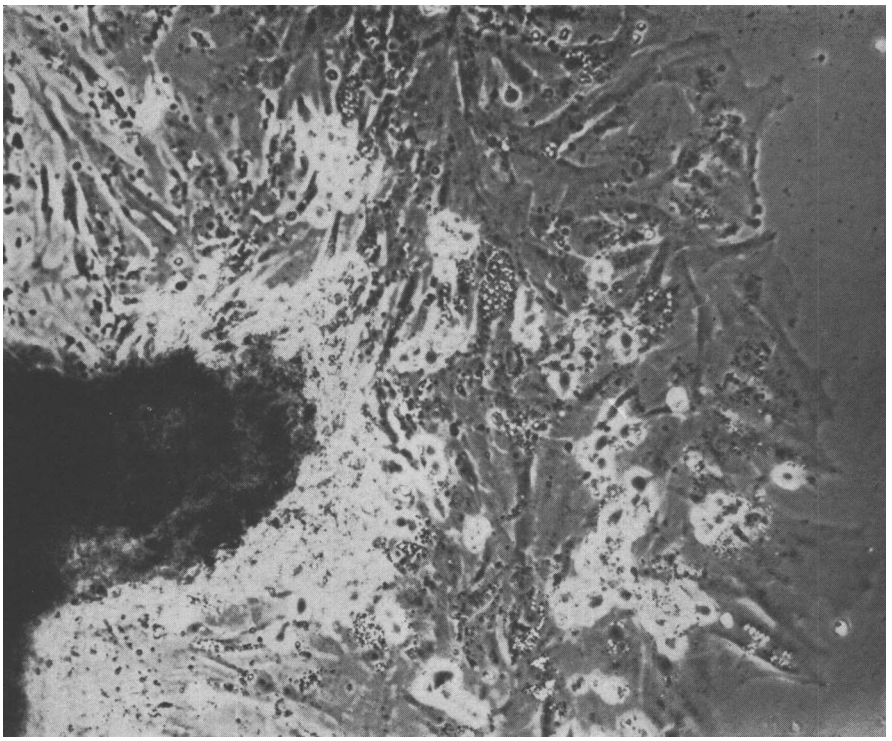

FIG. 1. Cellular outgrowth from human testicular tubule 10 days in culture.

a total of 103 cells were counted. Chromosome number varied from 8 to 46 with no apparent modal number. Two cells with over 80 chromosomes were observed in this culture. In Patient 14C, a total of 106 cells at metaphase were counted. Among these, only four cells had the normal diploid complement of 46 chromosomes. All of the remaining cells were hypodiploids (chromosome number range: 8–45). Since the chromosome loss in these two cultures were so extreme, a few experiments were designed to determine the effects of several factors which can induce nondisjunction (colcemid) (4), spread the chromosomes excessively or break the cells (centrifugation, flaming and hypotonic solution). The possible role of colcemid was examined by the following procedure using testicular culture derived from Patient 18C (2 mo in culture, 8th passage): Cells in the process of mitosis were removed from the flask by agitation in 1 ml Hanks' balanced salt solution (HBSS) and immediately 2 ml of distilled water was added. The cells were refrigerated at 4° for 2 min, centrifuged at 1,000 rpm for 5 min and fixed in 3:1 methanol:acetic acid. Slides were then made by air-drying and subsequently stained in Giemsa. Only those cells with unbroken cytoplasm were counted. In this instance, most of the chromosomes were intertwined with one another and could not be counted with absolute certainty. Of the 20 cells that could be scored, about 50% were hypodiploids with the chromosome number ranging from 38 to 44. The results showed that colcemid was not responsible for the hypodiploidy observed. The data were supported by the finding of hypodiploid cells in Patient 19C (one and one-half months in culture, 5th passage) grown on coverslips in multi-well plates (from Microbiological Associates, Bethesda), subjected to 1:2 growth medium:distilled water as hypotonic treatment and fixed without colcemid pretreatment.

In another experiment, which was designed to determine mainly the effect of hypotonic solution, the cells (Culture 19C) were grown on coverglasses in multi-well plates. As soon as the cells became monolayered, colcemid (0.4 μg/ml of medium) was added. Five hours later, the medium was poured off; the cells were fixed, air-dried and later stained. Most of the chromosomes at metaphase observed could not be counted. However, in those cells

which allowed a rough estimation, a proportion of them were hypodiploids. This experiment apparently provided the true chromosome picture of the testicular cells since no

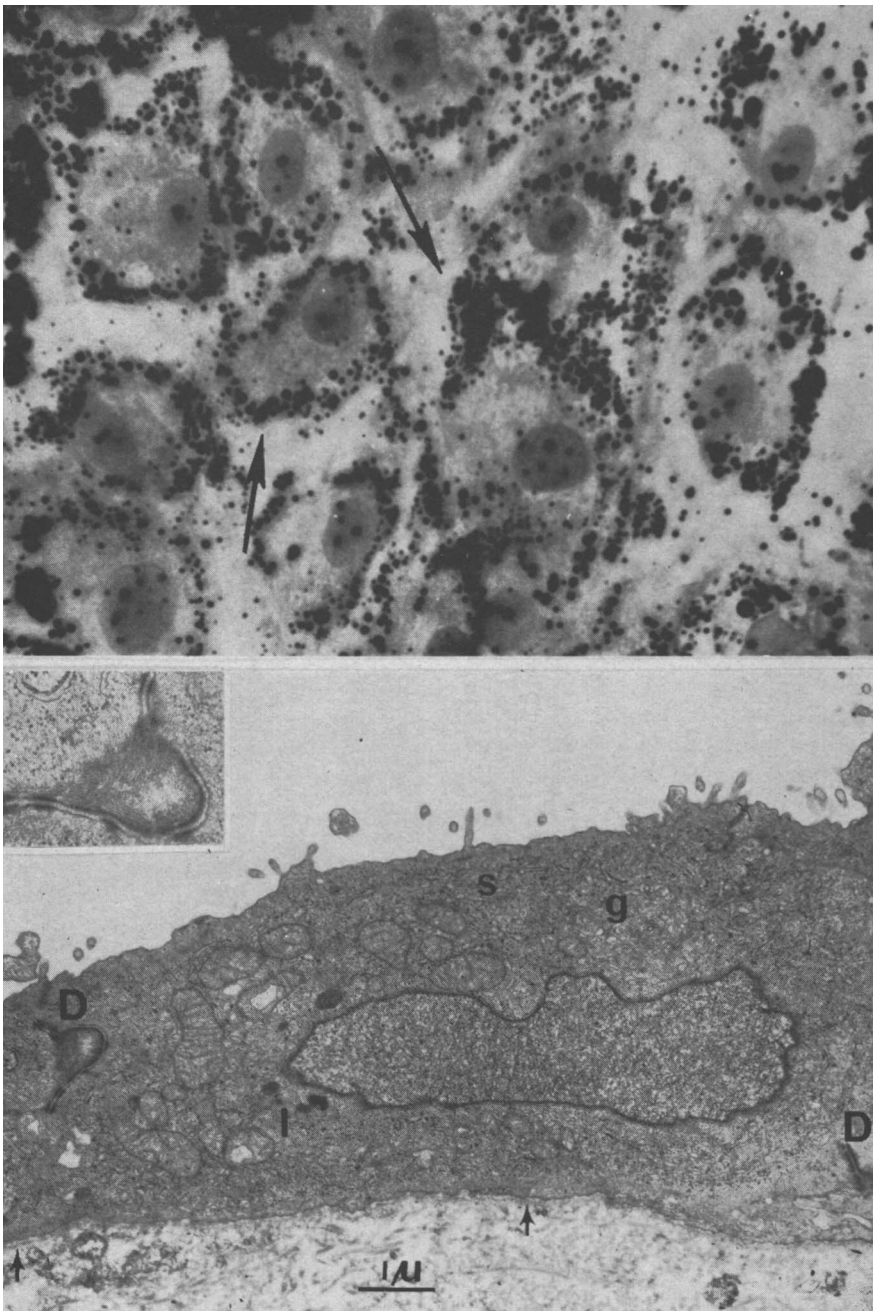

FIG. 2a. Epithelial-like cells stained with Sudan IV showing sudanophilic granules (arrows).

FIG. 2b. A cross section of an epithelial-like testicular cultured cell showing abundant smooth endoplasmic reticulum (s), Golgi complexes (g), mitochondria and lipid droplets (l). Also notice a basal lamina (arrows) resting under the cell. Desmosome type cell junctions are present between the cells (D & insert).

TABLE I. Distribution of Cells with Different Chromosome Numbers in Testicular Cultures.

No chromosomes per cell	No. cells in the designated culture			
	18C[b]	11C	19C	12C
10		1		1
11		0		0
12		0		1
13		0		0
14	1	0		1
15	0	0		0
16	0	0		0
17	0	0		1
18	1	0		0
19	0	0		0
20	0	0		0
21	0	0		0
22	0	0		0
23	0	0		0
24	0	0		0
25	0	0		0
26	0	0		0
27	0	0		0
28	1	0	1	0
29	1	0	0	0
30	0	0	1	1
31	0	0	0	0
32	1	0	0	0
33	0	0	0	0
34	0	0	0	1
35	0	0	0	3
36	0	1	4	2
37	2	2	0	4
38	2	2	5	6
39	0	2	1	3
40	2	4	3	3
41	5	4	1	4
42	4	4	5	7
43	5	3	2	6
44	11	7	4	4
45	16	3	2	4
46	76	27	17	12
ca. 75[a]	10	2	7	1
Total cells	138	62	53	65

[a] The exact chromosome numbers of these cells could not be determined with certainty. All cells appeared to have more than 75 chromosomes.

[b] One cell with 49 chromosomes.

centrifugation, no flaming and no hypotonic solution were used. However, such estimated chromosome counts cannot be regarded as an accurate assessment. In order to overcome this difficulty, the cells were grown according to the same method, using 1:2 growth medium: distilled water as the hypotonic solution; and it was found that most of the chromosomes within the cell could be counted. Subsequently, the experiment was repeated using cultures 18C, 19C, 11C (3 mo in culture, 10th passage) and 12C (4 mo in culture, 15th passage). Again the criterion for scoring was that only the cells with an unbroken cytoplasm were counted (Fig. 3a). In order to eliminate a bias in scoring the chromosome number, all those cells which presented an uncertainty in the near diploid range were not included. The results of all the chromosome counts are summarized in Table I. In Figure 3b, there were 18 chromosomes in one cell and 28 chromosomes in another. Karyotypes of these two cells indicated that the chromosomes combined constituted a normal chromosome constitution suggesting that the two cells arose from a common mother cell. The mechanism for this phenomenon is difficult to speculate, but can be the result of anomalous mitosis due to the failure of the separation of sister chromatids in the centromere region (5).

From Table I, it can be observed that the frequency of hypodiploids in Culture 18C is 38%, while that of Culture 11C is 53%, Culture 19C 55%, and Culture 12C 80%. The overall frequency of hypodiploid cells in these four cultures is 52%. Karyotyping of a large number of these cells processed by G-banding is now in progress in order to determine the pattern of chromosome loss.

Discussion. In our testicular cultures, we consistently observed chromosome loss in the six cultures studied. This is a unique phenomenon since it occurred in about 50% of the cells counted (Table I). The cause for the chromosome loss is not clear. It could not be completely due to the technical procedures since the results were obtained by several harvesting methods. The technical effect can be further ruled out because WI-38 cultures were found to have a normal diploid chromosome complement when they were harvested with the same methods and at the same time as the testicular cultures. In addition, our human blood cultures, domestic pig blood cultures and Syrian hamster cheek pouch cultures showed normal diploid modes.

In undescended testis, some hypodiploid cells with 45 chromosomes have been reported in a few patients (6). In carcinoma of the

cervix, hypodiploid stem lines have been observed as well as hyperdiploidy (1) which was very rare in our cultures. The fact that our cultures were initiated from patients with different clinical diagnoses (2 had hydrocele, one had cancer of the prostate, two had undescended testes and one had testicular feminization syndrome) rules out the possible association of hypodiploidy observed with a particular clinical condition of the patients. It should be noted that none of our cultures were derived from testes of normal persons. In testicular cultures derived from a normal domestic cat, our preliminary findings showed that

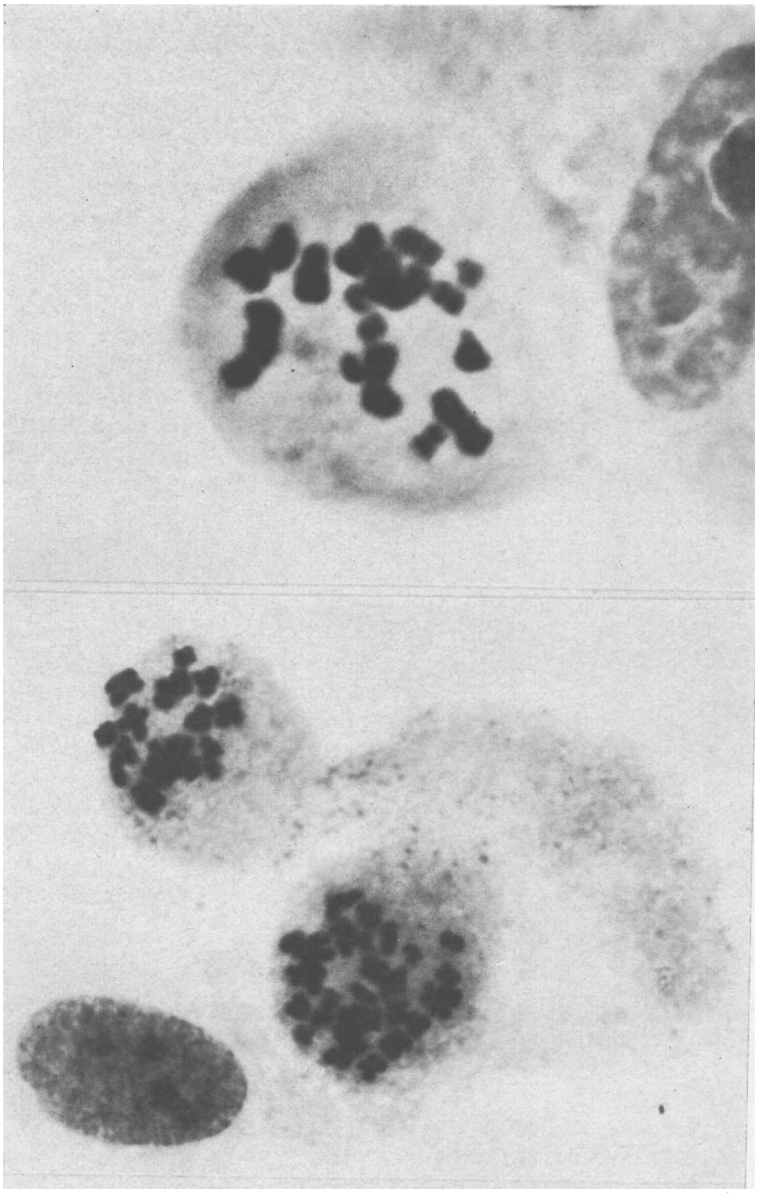

FIG. 3a. A human testicular cell with 17 chromosomes showing complete cytoplasm (from Culture 12C).

FIG. 3b. Two testicular cells with 18 and 28 chromosomes, respectively, (from Culture 18C) suggesting the possible origin of hypodiploidy in testicular cultures.

the chromosome constitution was normal. Conceivably, the chromosome loss observed in our human testicular cultures can be due to the possibly abnormal property of the testes. Nevertheless, the hypodiploidy of these testicular cells is potentially useful in genetic study, e.g., somatic cell hybridization, mutagenesis studies, as well as biochemical analysis.

Summary. Variable chromosome numbers with frequent hypodiploidy were observed in 6 human testicular cultures derived from testes of patients with diverse clinical conditions. The hypodiploidy can be due to the possibly abnormal properties of the testes.

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