Chromosomes of Human Endometrium

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Evidence is gradually accumulating about acquired somatic chromosome anomalies, particularly those of neoplasms. Unfortunately, control observations are almost entirely lacking. It is generally assumed that all the tissues of the body consist of cells with the normal karyotype as it is seen in cultured lymphocytes or fibroblasts, but this has not yet been shown to be the case. Even bone-marrow, which is rich in dividing cells, has not been the subject of extensive control observations to see how frequently there is a visible departure from the normal karyotype.

The present paper is a brief account of the findings in preparations of the chromosomes of human endometrial cells entering mitosis *in vivo*, where the tissue is histologically normal. Endometrium was chosen as the only accessible normal solid tissue with a sufficiently high mitotic rate.

Material and Methods

Endometrial curettings were obtained from the operating theatre. As soon as the material was removed it was placed by the surgeon in a solution consisting of one part of human AB serum (inactivated by heating at 56° C. for 30 minutes) and four parts of tissue culture medium 199 (Glaxo). It was processed within an hour of collection, or rarely two.

When the material arrived in the laboratory it was teased out with sharp-pointed forceps to separate epithelial cells from stroma, and the solid fragments were removed. The cell suspension was pipetted into a universal container and colcemid (Ciba) added to a final concentration of 1 mg./100 ml. It was incubated at 37° C. for 2 hours, with the object of improving chromosome contraction (it is not believed that any cells are found in metaphase which have not already begun division *in vivo*). The supernatant fluid was then replaced by 0.9% sodium citrate, and the suspension incubated for a further 10 minutes. It was then centrifuged again (5 minutes at about 750 rpm), and the deposit fixed with freshly made-up acetic acid-methanol (1:2). In a few cases the curettings were teased directly in 0.9% sodium citrate,

incubated at 37° C. for 10 minutes, centrifuged, and fixed in acetic-methanol.

Spreading was by either of two methods:

(1) (Cases 1-12 and 18-22) Material was left in fixative for several hours, centrifuged (5 min. at 1100 rpm) and resuspended in 50% acetic acid and left in the deep-freeze (-20° C.) overnight. Slides were cleaned by flooding with acetone and then water, and dried by wiping with tissue. A drop of cell suspension was then placed on the slide and gently warmed over a flame, allowing the drop to move over the glass surface until dry.

(2) (Cases 13-17) Material was stored overnight in acetic acid-methanol (1:3) at room temperature and the fixative changed several times before use. Slides were cleaned by flooding with acetone and then rinsed with cold tap water. A drop of cell suspension was squirted onto the slide and the same side gently flamed to burn off the alcohol and completely dried by waving in air.

Slides were stained with 1% lacto-acetic orcein (Harleco) and mounted permanently in Euparal.

Altogether 85 samples were processed, but only 22 could be used; the remainder showed few or no adequately displayed metaphases. Samples were accepted from various times in the menstrual cycle, and in some of the cases this was irregular. None of them, however, showed any morphological abnormality other than simple hyperplasia, either in sections or in Papanicolaou-stained smears from the curettage specimen.

Chromosomes of cells in metaphase were counted under the microscope, drawings being made either freehand or using a Wild drawing tube. Forty-six cells were analysed from photographs; this was particularly done whenever an abnormality was suspected, and (apart from chromosome loss) none was recorded unless it could be verified in this way.

Results

Fig. 1 shows an apparently normal cell from Case 2; it illustrates the best quality of chromosome spreading achieved with this method.

Table I shows the whole material giving the distribution of chromosome counts. Many cells were not included because they were obviously broken and incomplete, but it has to be admitted that this

FIG. 1. *Above*, a normal cell in metaphase from a direct preparation of human endometrium (Case 2). *Below*, the chromosomes of the same cell arranged as a karyotype.

judgement is very unreliable and the real extent of cell breakage is unknown. Doubtful counts are not included. Histograms of the chromosome counts from 12 cases are given in Fig. 2.

It will be seen that there is a large scatter of values to the left of 46, and the question arises whether the loss of chromosomes is random or not. Table II gives the Patau groups of the missing chromosomes from 41 cells in which this could be decided with

reasonable certainty. There seems to be a preferential loss of the E group chromosomes (16–18), which is difficult to explain by postulating any bias in the analysis.

Of the cells with 46 chromosomes, 51 were analysed. Only one showed a clear abnormality. It came from Case 3, and had an acentric fragment in place of a 17–18 chromosome. One other cell, from Case 11, appeared to have a deletion of the long

TABLE I

DISTRIBUTION OF CHROMOSOME COUNTS ON 279 CELLS FROM 22 ENDOMETRIAL SAMPLES

Case No.	Age (yr.)	Day of Cycle	Chromosome Counts													Histology and Comments
			< 35	35	36	37	38	39	40	41	42	43	44	45	46	
1	41		1										1	2	7	Non-secretory endometrium
2	34	14	8	1					1				2	6	7	Non-secretory endometrium
3	36		2	1				2	1	4		1		1	3	Early secretory endometrium
4	33	17	1											1		Early secretory endometrium
5	40												1			Non-secretory endometrium
6	48	7								2	1	3	3	9	20	Non-secretory endometrium
7	45	9									1		1	1	7	Non-secretory endometrium
8	34	16				1			3	1		3	2	4	21	Non-secretory endometrium, hypertrophic; amputation of cervix for carcinoma <i>in situ</i>
9	35	7	2		1		1		1	1			1	2	4	Non-secretory endometrium
10	25	8				1	1			1	1	1	1	2	4	Non-secretory endometrium
11	42				1			2	1	1	1	4	3	2	8	Non-secretory endometrium, hypertrophic
12	33										1					Secretory endometrium
13	33	15	1		1					3		2	1	4	8	Early secretory endometrium
14	51		1					1			1			3		Curetage after avulsion of fibroid polyp of cervix
15	31	10				1								2	4	Non-secretory endometrium
16	48	12												1	2	Non-secretory endometrium, hypertrophic
17	50	10													1	Non-secretory endometrium, hypertrophic; simple endometrial polyp
18	51		13	1	1		2				1	1		2	3	Non-secretory endometrium; post-menopausal
19	35	14	3									1			1	Decidua
20	43	29	3							1	1		1	2	4	Early secretory endometrium; small endometrial polyp
21	38	10	4								1	1			5	Non-secretory endometrium
22	34	13									1	1		1	1	Non-secretory endometrium
Total %			39 14	3 1.1	4 1.4	3 1.1	4 1.4	5 1.8	7 2.5	14 5	10 3.6	18 6.4	17 6.1	45 16.1	110 39.5	

arms of a No. 17 or 18 chromosome, but the quality of the metaphase was not quite good enough to allow this to be recorded with certainty.

Discussion

Until 1956 the methods in use for displaying human chromosomes were so unsatisfactory that the results are only of historical interest. In 1947 Barigozzi reported that squashed cells from normal endometrium had 48 chromosomes, then believed to be the normal human number. Timonen (1950) examined the chromosomes of endometrium from 41 patients not suffering from cancer. His specimens were fixed, Feulgen-stained, and squashed, and examined under phase-contrast. He found chromosome counts varying from 4–104, with the main peak at 21–25 and a lower one at 46–50. These results were challenged by Sachs (1954), who performed counts on 50 human endometrial cells and found no variations from the supposed normal diploid number. A scatter around a diploid peak was found by Walker and Boothroyd (1954), while Manna (1954) observed many values below the diploid mode but hardly any above.

After the introduction of hypotonic pre-treatment and the discovery of the correct chromosome number of man, it was found that cultured normal cells

from various organs showed a high constancy, with hardly any aberrations except for random chromosome loss (Levan and Bieseke, 1958; Tjio and Puck, 1958; Petursson and Fogh, 1963; Court Brown, Buckton, Jacobs, Tough, Kuenssberg, and Knox, 1966). Observations on direct preparations were less satisfactory, as the new techniques did not give a high success rate with suspensions made from most solid tissues. Ford (1964) has analysed the sources of error, and given reasons for believing that cells *in vivo* have an even higher chromosome constancy than cells in culture.

Human endometrium was examined by Takemura (1960), and of the 109 cells examined from five normal samples, 51% were found to have 46

TABLE II

CLASSIFICATION OF 86 MISSING CHROMOSOMES FROM 41 HYPODIPLOID CELLS

	1	2	3	B	C+X	D	16	17	18	F	G
No. of chromosomes lost											
Observed	0	1	3	6	32	8	6	18	8	4	
Expected	3.8	3.8	3.8	7.6	30.4	11.4	3.8	7.6	7.6	7.6	

Note: 'Expected' values are calculated from the proportions of the different chromosome groups present in the normal female set.

FIG. 2. Distribution of chromosome counts in direct preparations from 12 different samples of endometrium obtained by curettage. Counts below 30 have been ignored. Note the marked scatter to the left of 46, and the absence of hyperdiploid cells.

chromosomes. There was a scatter above as well as below this level. Wakonig-Vaartaja (1963) made counts on 428 cells from 16 patients, and 91% were found to have 46 chromosomes. Cells with less than 46 chromosomes amounted to 8%, and 1% had 47. 123 cells were analysed and 'the only variation from the normal karyotype was the presence of a few chromosomal deletions in 3 cells from one patient with endometrial hyperplasia'; the hyperdiploid cells do not appear to have been analysed.

Cultures of normal human endometrium have been examined by Hughes and Csermely (1965) with rather surprising results. Out of 2352 mitoses

studied, only 759 (32%) had the normal number of 46 chromosomes. There was a scatter of values not only beneath 46, but to a considerable extent above; for instance, 24 cells out of 449 were found with counts between 49 and 58 (Hughes and Csermely, 1966). The number with 47 is not given, because they are classified in this paper along with diploid cells in the group 44–48. These authors present several photographic karyotypes of normal or hypodiploid cells but none with supernumerary chromosomes.

Our findings resemble those of Hughes and Csermely in the high frequency of random chromosome loss, but not at all in the matter of cells with supernumerary chromosomes. The simplest explanation for the occurrence of a substantial number of hyperdiploid cells in other series is that cells have been included which do not have the chromosomes displayed well enough for a really exact count, as well perhaps as the presence of acentric fragments counted as chromosomes. An additional explanation of the difference between our findings and Hughes and Csermely's is that aneuploidy may develop in culture to an extent not observed *in vivo*.

Because of technical difficulties, we do not believe that the last word has been said on this question, and in particular we need to know whether the hypodiploid cells found in such large numbers are really present in the living endometrium, and, if they are, what is the mechanism of chromosome loss. Assuming that chromosome loss is due in some way to the technical procedure, the findings in our series support Ford's (1964) view that there is hardly any departure from diploidy and that spurious counts explain some of the variation recorded.

Summary

Direct preparations were made to examine the chromosomes of non-malignant human endometrium. Out of 85 samples, 22 furnished metaphases which were suitable for chromosome counting. Whenever possible a karyotype analysis was also done. The modal chromosome number was 46 but there was a large scatter of values to the left due to chromosome loss. Cells with more than 46 chromosomes were not encountered, and those with 46 chromosomes had apparently normal karyotypes.

This work was supported by the British Empire Cancer Campaign. The authors are very grateful to Mr. J. A. Stallworthy and his staff in the Department of Obstetrics and Gynaecology, Churchill Hospital, for supplying the samples of endometrium.

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