tradiol-6, 7 -^{3}H (EE) or ethynylestradiol-6, 7 -^{3}H-3-cyclopentyl-1-^{14}C ether (EECPE) were killed at 7.5-, 30- or 120-min intervals. Intestinal lumen contents, intestinal wall homogenates and portal plasma were analyzed for free and conjugated steroids. After 7.5 min, more than 80% of the administered dose of E or EE had been absorbed. Characterization of the steroids present in the various ether extractable fractions (free steroid) indicated that, unlike EE, E had undergone rapid metabolic transformation with estrone accounting for 70% of the total radioactivity in the intestinal wall and portal blood. This transformation to the less potent estrone could be responsible for the reduced biological activity of orally administered E.

The intestinal absorption of EECPE was markedly different from that of E or EE. The EECPE was slowly absorbed from the lumen through the intestinal wall. At 7.5 min as much as 85% of the administered dose was still present. Thereafter, the compound was slowly transported with almost complete absorption of the free steroid by 120 min. This delay may be due to binding of EECPE to the fatty material present in the intestinal wall. For a truer evaluation of intestinal absorption, an analysis of whole homogenized intestine may be required to determine whether it significantly exceeds the residual amount of drug present in the intestinal lumen.

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Karyotypic Changes in Mycoplasma-Modified Lines of FL Human Amnion Cells* (33464)

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Chromosome changes in mycoplasma-infected FL human amnion cells have included reduction in chromosome numbers, increase in the frequency of aberrations, and the appearance of several new chromosome varieties (1). The very slowly developing changes continued even during the second and third year of cultivation of the infected cultures, as previously reported (2). After elimination of mycoplasma the chromosome numbers did not revert to higher numbers, and one new chromosome, a large telocentric, persisted in practically all metaphase plates (2). There have been correlated changes in morphology, cultivation characteristics, and in resistance to mycoplasma. The tumor-producing capacity was reduced when the cells were tested in the cheek pouch of cor-

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tisonized weanling hamsters. This reduction was evident both before and after mycoplasma elimination (3).

Changes in the karyotypes of cells in the mycoplasma-infected line of FL cells (F 138) and in cells of this line from which mycoplasma were eliminated (F 138 cl) are presented in this report.

Materials and Methods. Cells. FL line of human amnion (4); F 138, which is a line (1) of FL human amnion cells exposed to mycoplasma (strain HT) (5), cultured as mycoplasma-infected for approximately 3 years; F 138 cl, the designation for cells of line F 138, which after 811 days of culture in the presence of mycoplasma were exposed for a short period to Aureomycin treatment. Mycoplasma were eliminated, and the cell line has subsequently been cultured for 1 year in the absence of mycoplasma (2). The cell lines were cultured in LY medium containing 20% human serum (4), except for line

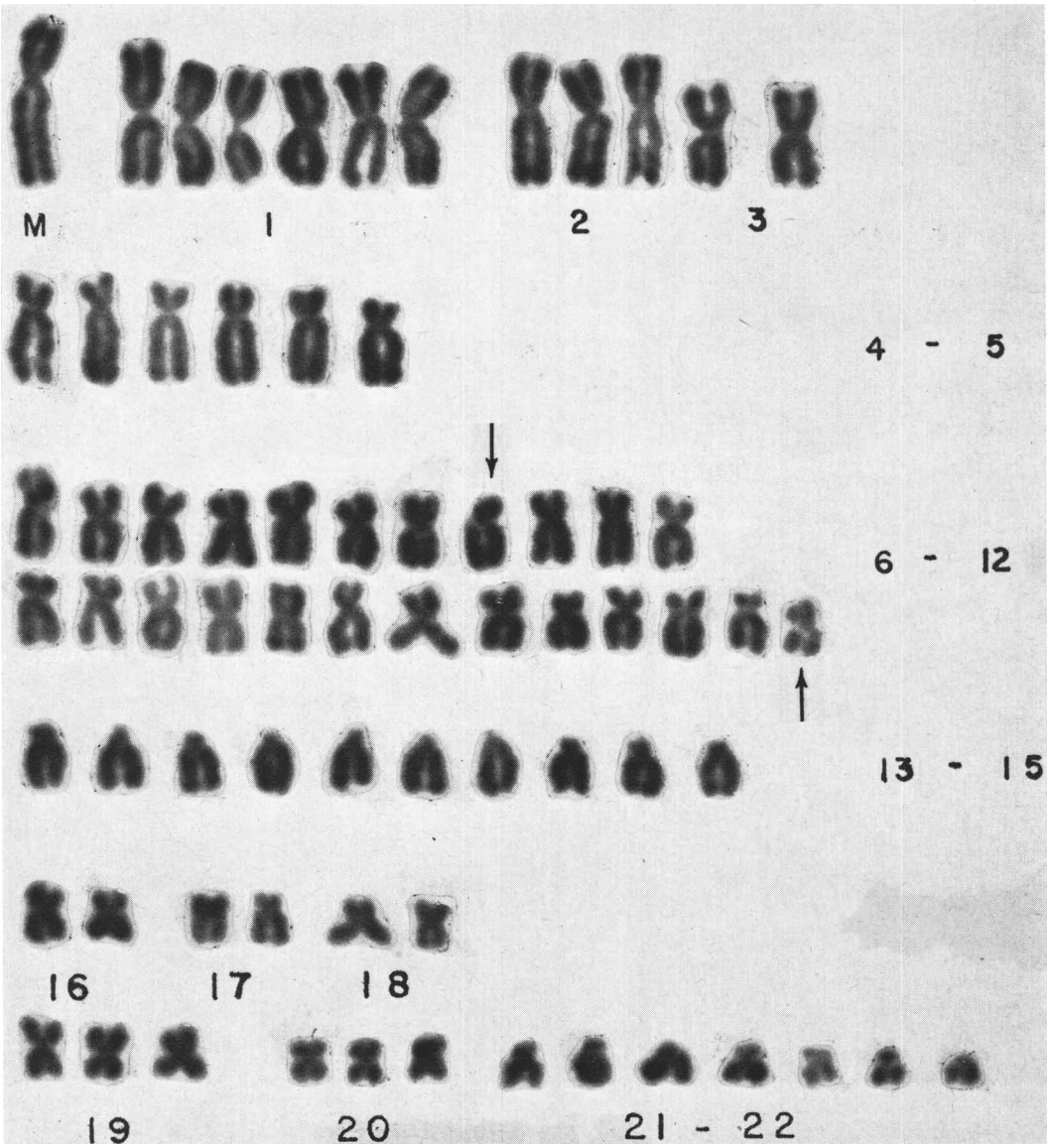

FIG. 1. Karyotype of FL human amnion cells; 71 chromosomes.

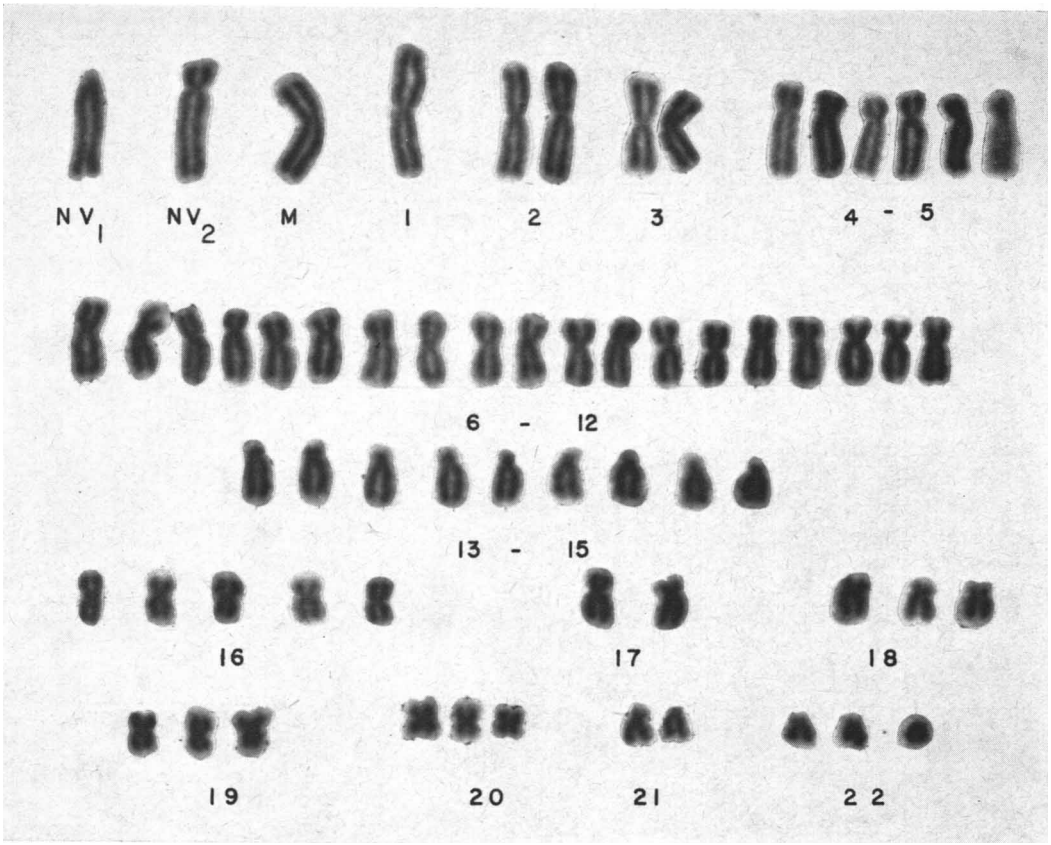

FIG. 2. Karyotype of F 138, a mycoplasma-carrying cell line derived from FL cells; 63 chromosomes.

F 138 cl, which for a short period during the Aureomycin treatment was cultured in medium 512 (6) containing 15% fetal bovine serum. It was shown in control experiments that Aureomycin and the medium change as such caused no changes of the chromosomes. The cells have been transferred weekly by trypsinization. They have been fluid changed according to needs; the mycoplasma-infected cultures were, as a rule, fluid changed daily.

Results. There was considerable variation among metaphase plates within the three examined cell lines. The chromosomes from 10 metaphase plates of each cell line were arranged according to the Denver classification (7). A karyotype from each line is shown in Figs. 1–3. In the FL cell karyotype, shown in Fig. 1, there are 71 chromosomes, of which one is the very large submetacentric marker (M), present in all FL cells. The human karyotype can still be recognized, but in comparison with the normal human karyotype there are additional chromosomes in most of the groups. Thus, in this cell there are 6 chromosomes No. 1; 3 No. 2; 2 No. 3; 6 in group B; 24 in group C; 10 in group D; and 6 in group E. In group F there are 6 chromosomes, and there are 7 in group G. A characteristic feature of FL cells is the marked secondary constrictions in two chromosomes in the C group (arrows).

The karyotype shown in Fig. 2 represents the F 138 line. In this karyotype with 63 chromosomes there is 1 No. 1 chromosome; 2 No. 2; and 2 No. 3 chromosomes. The marker chromosome, as well as the 2 new chromosome varieties, the long telocentric chromosome (NV_1) and the large subtelocentric (NV_2) are seen. There are 6 chromosomes in

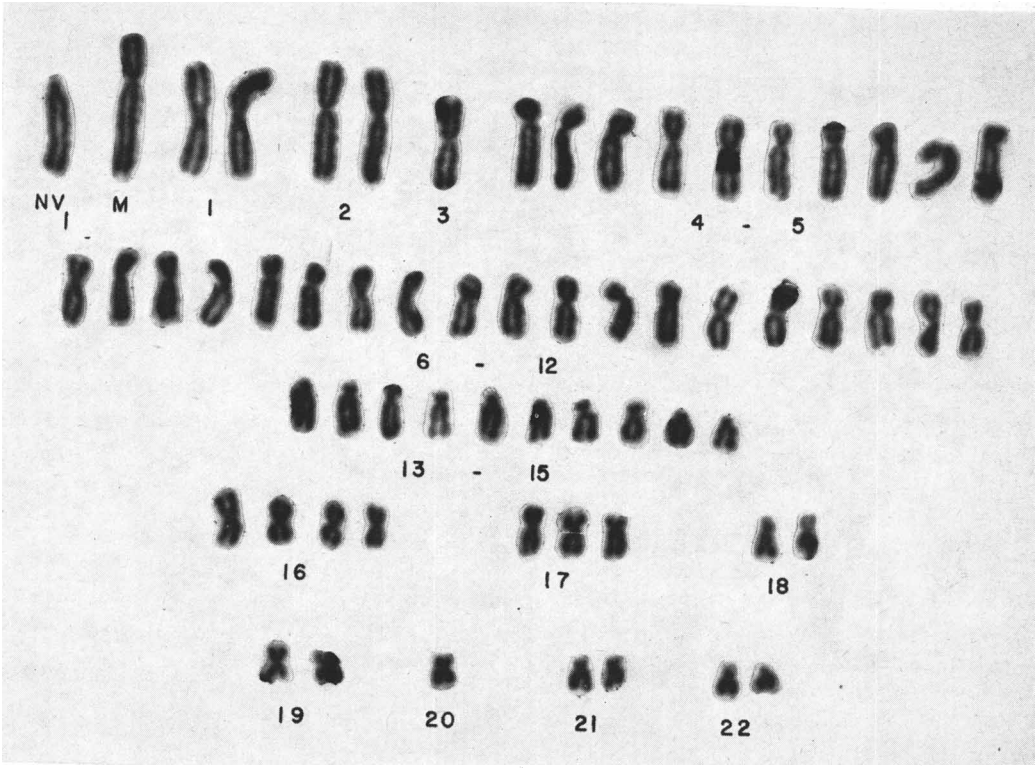

FIG. 3. Karyotype of F138 cl, the designation for the F 138 cell line after elimination of mycoplasma; 62 chromosomes.

the B group; 19 in the C group; 9 in the D group; 10 in the E group; 6 in the F group; and 5 chromosomes in the G group. The 2 chromosomes with marked secondary constrictions in the C group were not observed in this or any other examined metaphase plates of the F 138 line.

Figure 3 represents line F 138 cl. In this karyotype with 62 chromosomes the new variety NV_1 and the marker chromosome are also present. There are 2 No. 1; 2 No. 2; only 1 No. 3 chromosome, but 10 chromosomes in the B group. There are 19 chromosomes in group C; 10 in group D; 9 in group E; only 3 in group F; but 4 in the G group. The 2 chromosomes with marked secondary constrictions were also absent in metaphase plates of this cell line.

The distribution of chromosomes per group for all 10 analyzed metaphase plates of each of the three cell types is shown in Table I. It is notable that of the 10 analyzed FL cells 5 had one marker chromosome, 4 had two, and 1 had even three marker chromosomes. The new chromosome variety NV_1 is present in all the karyotypes of mycoplasma-modified cells before and after mycoplasma elimination; new variety NV_2 occurred in 4 of 10 F 138 cells, but not in these karyotypes of F 138 cl cells.

Mean values of the distribution of chromosomes per group for the 10 karyotypes of each of the three cell types are shown in Fig. 4. The figure shows, for comparison, the number per group in a diploid human cell. The X chromosome is included in the C group; the Y chromosome in the G group. In FL cells there is increase in the average number of chromosomes for all chromosome groups, most for chromosome No. 1 (80%), group D (68%); chromosome No. 3 (60%), and group C (54%), in comparison with the number per group in a diploid cell.

The mean values for mycoplasma-infected F 138 cells differ obviously from FL cells. All chromosome groups contain fewer chromo-

TABLE I. Number of Chromosomes in Individual Chromosome Groups for 10 Karyotypes of Each of the Cell Lines: FL, F 138, and F 138 cl.[a]

Type of cell	Karyotype no.	Chromosome groups, marker (M), and new varieties (NV_1 and NV_2)											
		A			B	C	D	E	F	G			
		1	2	3	4–5	6–12	13–15	16–18	19–20	21–22	M	NV_1	NV_2	Total
FL	1	4	3	3	6	20	12	7	6	6	1	—	—	68
	2	3	3	3	6	22	12	7	5	6	2	—	—	69
	3	3	4	2	6	21	11	11	4	6	1	—	—	69
	4	3	3	3	6	24	10	5	5	9	2	—	—	70
	5	4	2	4	6	21	10	8	6	7	2	—	—	70
	6	2	3	4	6	24	10	9	6	5	2	—	—	71
	7	6	3	2	6	24	10	6	6	7	1	—	—	71
	8	5	3	3	6	25	8	7	6	7	1	—	—	71
	9	3	3	3	5	27	8	9	5	5	3	—	—	71
	10	3	4	5	4	23	10	10	5	7	1	—	—	72
F 138	1	2	3	2	8	17	7	9	5	6	1	1	—	61
	2	2	2	2	7	17	8	9	8	5	1	1	—	62
	3	2	2	2	6	21	8	9	6	4	1	1	—	62
	4	1	2	2	6	19	9	10	6	5	1	1	1	63
	5	1	2	3	7	16	10	9	7	7	1	1	—	64
	6	2	1	2	8	19	8	6	6	9	1	1	1	64
	7	2	2	2	8	20	9	5	7	6	1	1	1	64
	8	3	3	2	6	18	7	6	8	8	1	1	1	64
	9	2	3	2	6	18	8	8	8	7	1	1	—	64
	10	2	2	2	6	22	10	6	5	7	1	1	—	64
F 138 cl	1	2	3	2	9	21	8	6	3	4	1	1	—	60
	2	3	2	2	10	18	10	5	4	6	1	1	—	62
	3	2	2	2	10	18	9	8	5	4	1	1	—	62
	4	2	2	2	11	19	9	5	5	5	1	1	—	62
	5	2	2	2	8	17	10	6	5	8	1	1	—	62
	6	2	2	1	10	19	10	9	3	4	1	1	—	62
	7	2	2	2	10	22	9	6	4	3	1	1	—	62
	8	1	3	2	9	20	7	9	5	6	1	1	—	64
	9	1	2	3	9	24	10	5	3	5	1	1	—	64
	10	1	2	4	9	20	8	8	5	5	1	1	—	64

[a] M = the large submetacentric marker chromosome characteristic for FL cells; NV_1 = large telocentric new variety; and NV_2 = large subtelocentric new variety; both of these chromosomes observed only in the mycoplasma-modified cell lines.

somes than FL cells, except for an increase in group B and group F (70% and 40%, respectively, compared to a diploid cell). The mean numbers of Nos. 1, 2, and 3 chromosomes are reduced to the approximate level of that in a diploid cell. The numbers in the remaining groups are increased from the diploid level.

The F 138 cl karyotypes show similarities to F 138 karyotypes in groups A, C, and D. The mean numbers of chromosomes in these three groups are less than those for FL cells. In group A the numbers for F 138 cl are close to the diploid cell level. This is also the case for groups E, F, and G, in which the chromosome numbers are clearly less than the numbers for both FL and F 138 cells. A definite increase (138% over the number in the diploid cell) has occurred in F 138 cl cells in group B. The mean value for this group is greater than the value for FL, as well as for F 138 cells.

Discussion. The karyotypes of the three cell lines were studied to compare their indi-

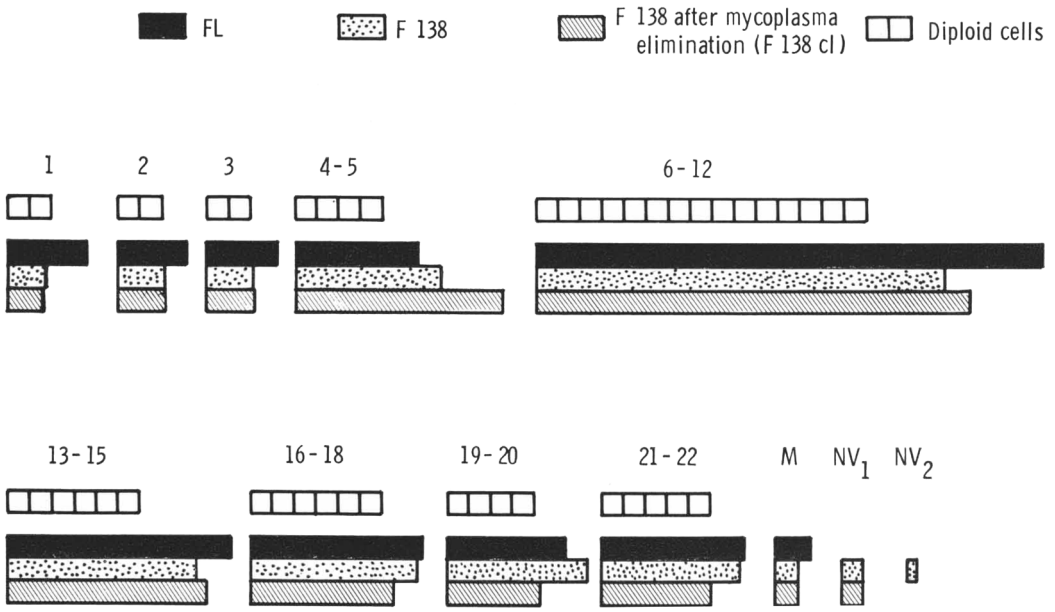

FIG. 4. Mean values of chromosomes per group from 10 karyotypes/cell type.

vidual features in an attempt to correlate specific chromosome changes in mycoplasma-modified FL cells with other observed biological changes. Metaphase plates with well spread-out chromosomes and with complete outline were chosen for the karyotypes. Plates containing abnormalities, as endoreduplication, dicentric chromosomes, acentric fragments, minute chromosomes, translocational exchanges, ring chromosomes, chromatid breaks, and chromosome destruction, abnormalities which have been observed in the three cell lines at various frequencies (2), were avoided.

The variation in number of chromosomes per group is in line with the variability of other features of malignant cells in contrast to normal cells, for example, the variation in cell size and shape within a malignant cell population. The variation within the 10 karyotypes of each cell line is not greater, however, than to permit the establishment of the mean values demonstrated in Fig. 4. Hence, the differences in these mean values could be discussed as being possibly correlated with other biological differences among the lines.

As described here, and in general agreement with an analysis reported 5 years previously (8), FL cell karyotypes are profoundly changed from the karyotype of the normal human cell. They contained high numbers of chromosomes, reflected in an increase in the number of chromosomes in all groups. The marker chromosome, two chromosomes with marked secondary constrictions, and various aberrations (not indicated in the 10 karyotypes presented here), were all characteristic of this cell. The FL cells are rapidly growing cells (4) with morphological and other *in vitro* malignant features; they are capable of producing tumors (3, 9, 10). The mycoplasma-infected and -modified F 138 cells in contrast have a lower division rate *in vitro,* they show more variation in cell size and shape, with not infrequently appearing giant cells, and the resistance to superinfection with mycoplasma is increased. Their tumor-producing capacity is reduced. The F 138 cl cell line is closely similar to the F 138 in tumor-producing capacity, as judged by the number of cells necessary for tumor production and from average tumor sizes (3); resistance of this cell line to mycoplasma infection is also increased. Its growth rate *in vitro,* however, approaches that of FL cells. Both mycoplasma-modified lines have reduced staining affinity (hematoxylin and eosin, acridine orange) when compared to FL cells, but F 138 cul-

tures in contrast to F 138 cl continuously carry mycoplasma infection in the culture supernatant and as a heavy coating of cell-associated microorganisms (11).

It is conceivable that at least some of the chromosome differences are related to the other observed differences among the cell types. However, whether the most apparent or the less conspicuous chromosome changes are most important (or perhaps even changes that cannot be observed with the present techniques) cannot be settled at this time. The interpretation, necessarily, must be theoretical, pending further studies for more conclusive evidence. It is tempting to propose that malignancy, as expressed by the degree of tumor-producing capacity, is directly correlated with total chromosome number, as our present results suggest. It may also be inferred from the data that the increase of chromosome aberrations observed in F 138 as compared to F 138 cl cells, is insignificant in regard to tumor-producing capacity (number of cells necessary for tumor production), inasmuch as the two cell lines are similar in this respect. It is conceivable, however, that a higher degree of necrosis, which was observed in tumors originating from the mycoplasma-infected F 138 cells, could be related to the higher proportion of cells with chromosome aberrations in this cell line, although the necrosis more likely is an effect of the mycoplasma infection as such. The role of the large telocentric new chromosome, characteristic of and present in nearly all cells of the mycoplasma-modified lines F 138 and F 138 cl, is unknown at this time, However, a recently developed modified FL line (FL-H-F 138) has maintained high chromosome numbers and contains this large telocentric chromosome at a high frequency. Further studies of this line and others which have also changed more specifically in chromosomal respect as compared to the lines reported here, could reveal more conclusive information on the possible correlation between mycoplasma-induced chromosomal and other biological changes in regard to malignancy.

Summary. Karyotypes of the mycoplasma-infected and -modified F 138 line of FL human amnion cells and of this line after mycoplasma elimination (F 138 cl) differ distinctly from karyotypes of FL cells. In the hypertriploid FL cells there is an increase in number of chromosomes in all groups as compared to the diploid cell (Denver classification). All FL metaphase plates contain a large submetacentric marker chromosome, sometimes in duplicate or triplicate, and two group C chromosomes with marked secondary constrictions. The hypotriploid F 138 and F 138 cl karyotypes differ from FL by containing fewer chromosomes in groups A, C, and D, but more chromosomes in group B; the two group C chromosomes with pronounced secondary constrictions are absent, but notable new chromosome varieties have appeared. The F 138 cl karyotypes contain lower number of chromosomes in groups E, F, and G, but higher numbers in group B than F 138 karyotypes. There is pronounced variation among karyotypes within each cell line. Attempts are made to correlate differences among karyotypes of the three cell lines to their differences in biological behavior *in vitro* and *in vivo*.

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