Aneuploidy and Age in a Population Survey

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THE MAGNITUDE and frequency of deviation from the diploid chromosome number in normal human subjects have not, as yet, been firmly established. In most published reports, the control populations consist of laboratory or patient groups thought to be physically normal. The incidence of aneuploidy has been estimated recently (Jacobs *et al.*, 1961, 1963, 1964; Hamerton *et al.*, 1965; Court Brown *et al.*, 1966), and the results indicate an association between hypoploidy and age, particularly in female subjects over 65 years of age. Since selection bias may be present in these studies, we undertook to investigate the chromosome constitution of a statistically determined probability sample of the population of the metropolitan area of Buffalo, New York. Such a population would yield information on the distribution of deviations from the diploid number in an unselected population and provide data for comparison with other groups of subjects. In addition, the collection of these data from an urban population in the United States affords a comparison with populations in other parts of the world and with cytogenetic data to be obtained in the future.

MATERIALS AND METHODS

The subjects in this study were selected from the Buffalo metropolitan area by a sampling procedure designed for a leukemia survey described in detail elsewhere (Graham *et al.*, 1963). The random sample was based on a stratified selection of households within census tracts prior to the collection of blood samples. The selection of the individual in the house was governed by a predetermined sampling procedure to yield the proper number of children and adults necessary. The examined group consisted of 171 subjects, 99 females and 72 males.

Heparinized blood was obtained from the selected individuals and leukocyte cultures were initiated according to the method of Moorhead *et al.*

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(1960) with slight modifications (Sandberg *et al.*, 1962). Cells were in culture for a total of 72 hours, and colchicine (0.001 μg./ml.) was added for the last hour. It was hoped to examine 100 metaphases per patient, but this was not possible in all cases. A total of 10,393 cells was counted, with an average of 60.8 cells per person.

The examination of the coded slides was performed by three observers. Each cell was counted at least three times, thereby verifying the chromosome number. In addition to the number of chromosomes, observations were made on the number of small acrocentrics, the presence or absence of satellites on the acrocentrics, breaks, tetraploidy, endoreduplication, and obvious structural aberrations of chromosomes. Analysis of each metaphase was performed microscopically and counts verified photographically when indicated.

RESULTS

The data were analyzed in two ways; the first method is similar to that of Jacobs *et al.* (1961, 1963) and Hamerton *et al.* (1965), in which subjects were grouped into nine age classes; the second method utilized the data for each subject individually (ungrouped), thus allowing a closer examination of the *degree of relationship* between age and aneuploidy (expressed as the percentage of hypodiploid or hyperdiploid cells to the number of diploid cells).

In this paper, the degree of relationship is of central importance for the comparison of these data with others. Therefore, a brief explanation follows. The degree of relationship refers to that proportion or percentage of the variability in the dependent variable (percentage hypo- or hyperdiploid cells) that is associated with the variability in the independent variable (age). A high proportion (for example, 0.8 to 1.0) suggests a strong relationship, whereas a low proportion (for example, 0.00 to 0.20) suggests a weak relationship. It is possible to have a statistically significant relationship, but yet have a weak relationship. For example, if the proportion were 0.15 (and significant), this would indicate that most of the variability in hypodiploidy, 1.00 − 0.15 = 0.85 or 85%, is unexplained. When the degree of relationship is low, this would indicate that the independent variable (age) contributes little to the understanding of the dependent variable (hypodiploidy).

Grouped Analysis

The data in Table 1 are classified into the same nine age groupings used by others (Jacobs *et al.*, 1961, 1963; Hamerton *et al.*, 1965). That these data appear somewhat different from an expected random distribution of ages was due to the presence of insufficient numbers and the necessity to combine some age classes (e.g., 45–64 for females). The mean age for each group was used in the calculations rather than a weighted mean since it closely approximated the mean age weighted for the number of cells counted. The relationship between percentage of both hypodiploid and hyperdiploid (exclusive of polyploid) cells and age was studied, using the least squares technique.

TABLE 1. TABULATION OF CHROMOSOMAL COUNTS BY AGE AND SEX

Age group	No. persons	Mean age	Total no. cells counted	No. of cells with counts of			Hypomodal count, % of modal count	Hypermodal count, % of modal count
				<46	46	>46		
Females								
0–14	23	8.8	1870	98	1751	21	5.60	1.20
15–24	12	18.2	783	42	731	10	5.75	1.37
25–34	8	28.1	415	23	390	2	5.90	0.51
35–44	16	38.3	1231	82	1135	14	7.22	1.23
45–64	25	53.0	1403	71	1319	13	5.06	0.99
65–74	10	68.7	754	57	689	8	8.27	1.16
75+	5	77.2	172	20	152	0	13.16	0.00
TOTAL	99		6628	393	6167	68	6.37	1.10
Males								
0–4	2	2.5	139	9	128	2	—	—
5–14	13	8.7	789	57	722	10	7.89	1.39
15–24	12	17.5	642	39	591	12	6.60	2.03
25–34	14	29.7	718	44	669	5	6.58	0.75
35–44	8	38.6	410	34	373	3	9.12	0.80
45–54	9	49.8	437	31	403	3	7.69	0.74
55–64	9	61.2	411	26	380	5	6.84	1.32
65–74	4	69.2	206	9	195	2	4.62	1.03
75+	1	77.0	13	1	12	0	—	—
TOTAL	72		3765	250	3473	42	7.20	1.21

Regressions on age: males hypomodal $Y = 8.14 - 0.028X$; males hypermodal $Y = 1.50 - 0.009X$; females hypomodal $Y = 3.89 + 0.081X$; females hypermodal $Y = 1.35 - 0.010X$.

The linear regression equations for hypodiploid and hyperdiploid percentages of the two sexes are given in Table 1. The calculated linear and quadratic coefficients were not statistically significant for the males. The coefficients for hyperdiploid percentages for the females was not significant. There was a significant ($P < .05$) quadratic relationship for the hypodiploid counts among the females. The single, very high percentage (13.16) for the oldest females was largely responsible for the significant curvilinear relationship. If hypodiploidy percentage is unusually high only among older females, then the grouping by ages, as used here, is not the most sensitive method to examine the degree of the relationship.

A comparison of the results of Jacobs *et al.* (1963), Hamerton *et al.* (1965) and the present survey reveals substantial evidence of a relatively high incidence of hypodiploid cells among older females (Fig. 1). However, there was no consistent and regular increase in hypodiploidy between 0 and 60 years of age in these data and the data of Hamerton and coworkers.

The absence of an age-hyperdiploid association in both Table 1 and in the results reported by Hamerton *et al.* (1965) does not support the findings of Jacobs *et al.* (1963). In fact, the findings in a subsequent report by Court Brown *et al.* (1966) were not consistent with their results published in 1963

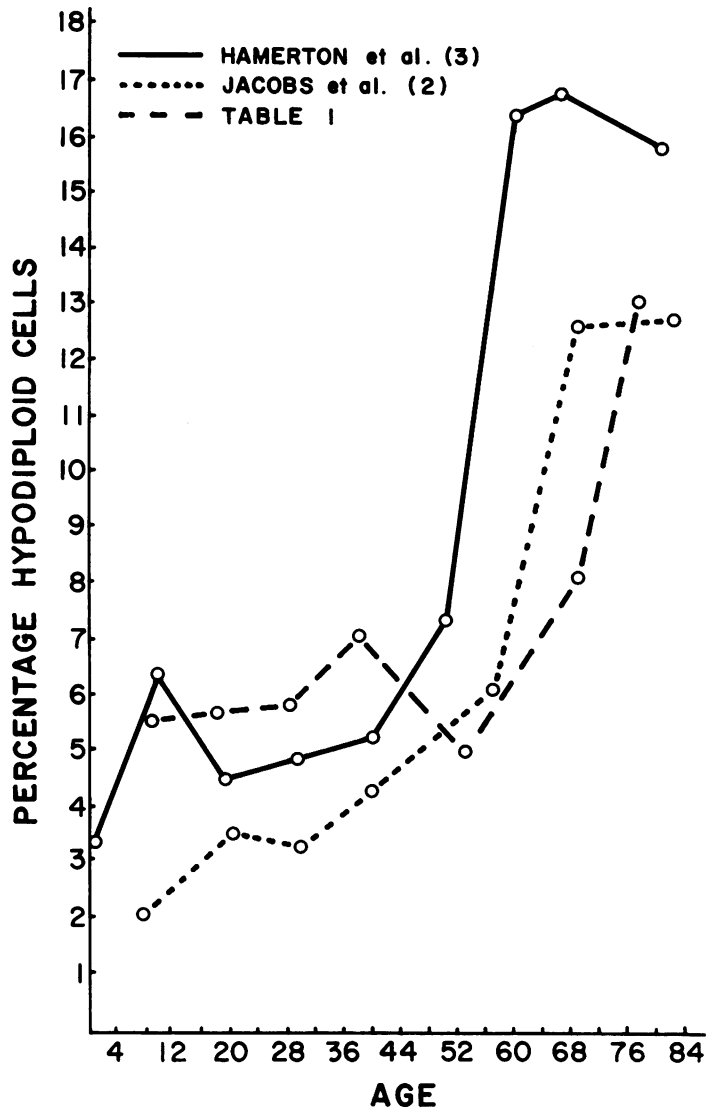

FIG. 1. Comparison between the relationship of percentage hypodiploid cells and age for females.

(Jacobs *et al.*, 1963). In the first report, the hyperdiploid percentage for 25 subjects over 74 years of age was 4.2% (Jacobs *et al.*, 1963), whereas the percentage was 1.5% for 62 subjects in the second report (Jacobs *et al.*, 1964).

Ungrouped Analysis

An analysis of the data as grouped in Table 1 offers no way of determining the contribution of a single person to a particular age group, since the number of cells counted per individual could influence the mean of an age group. A subject with many more cells counted than the others in the group would have

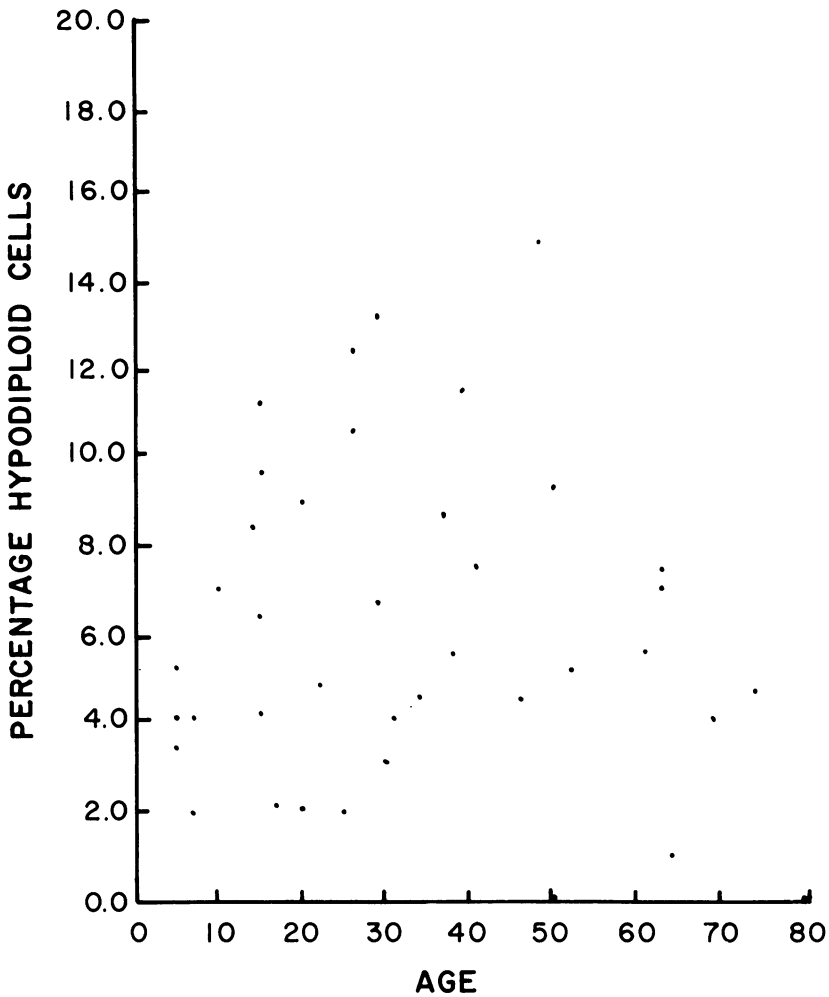

FIG. 2. Relationship between age and percentage of hypodiploid cells for 41 males with more than 39 diploid cells counted.

a disproportionate effect on the percentage for the particular age group. For this reason and also to obtain an estimate of the degree of relationship, a least squares analysis was performed using the age and percentage of aneuploidy for each subject individually.

Instead of the nine age classifications, a total of 171 ages and percentages, or one pair for each person, was used. Such an analysis did not suggest that the incidence of aneuploidy increased with age for males or females. In fact, a significant ($P < 0.05$) negative relationship was obtained between hyperdiploid percentage and age when the 171 subjects were considered. However, the correlation between age and hyperdiploid percentage was only -0.13, indicating that only about 2% of the variability in the percentage was associated with age.

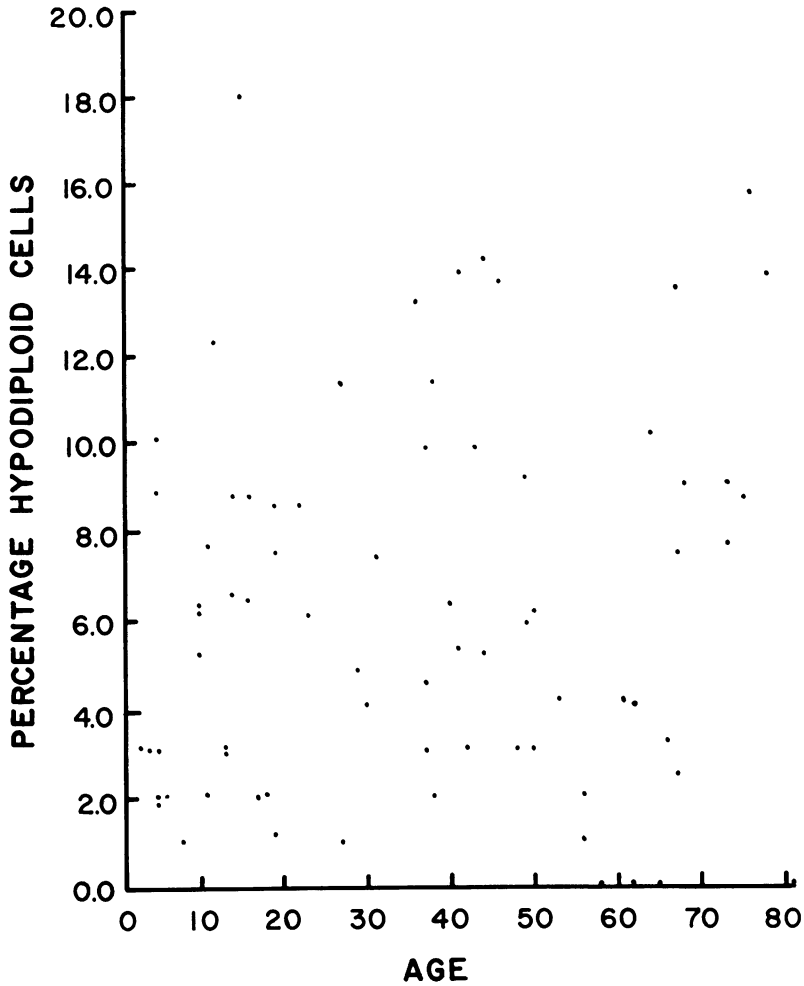

FIG. 3. Relationship between age and percentage of hypodiploid cells for 71 females with more than 39 diploid cells counted.

The data were refined to include only subjects with more than 39 diploid cells. This grouping eliminates most of the difficulties associated with percentage based on small numbers. A graphic representation of these data (Figs. 2, 3) discloses no association between age and percentage of hypodiploid cells. In addition, there were no statistically significant linear or quadratic regression coefficients. However, it is noteworthy that all but one of the females above 68 years of age exhibited greater than 8% hypodiploid cells (Fig. 3). It is this group which contributes to the high hypomodal percentages in the older females (Table 1).

A recent analysis by Court Brown *et al.* (1966) of new data and their earlier reported data (Jacobs *et al.*, 1963) suggests that the relationship between age and aneuploidy is not as clear-cut as originally indicated. An ungrouped analysis of their data (Fig. 4) indicated an abrupt change in the

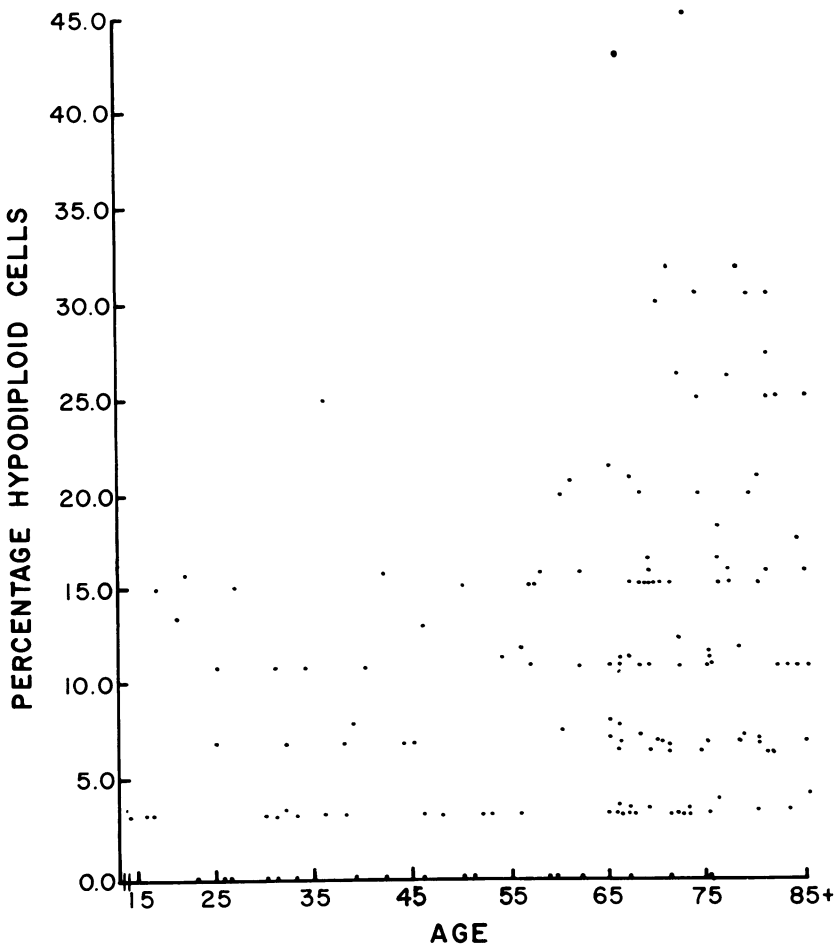

FIG. 4. Relationship between age and percentage of hypodiploid cells for 164 females with more than 14 diploid cells counted from the series of Court Brown *et al.* (1966).

distribution of percentages for females over 60 years of age. The data in Fig. 4 show the percentage of hypodiploid cells (based on percent of diploid cells) for 164 women in their sample with more than 14 diploid cells counted. Only cells with a 65+ hours culture time were used. Although a quadratic curve was significant ($P < 0.01$), the degree of relationship was low; only 7% of the variability in hypodiploidy was associated with age.

Other Observations

An analysis was made to determine whether age, diploid counts, hypodiploid counts, and hyperdiploid counts were interrelated such that the association between age and percentage might be affected. There was no evidence of interrelationships among factors.

Table 2 contains the data on chromosome breaks, tetraploidy, endoreduplication, and hyperdiploidy. Table 3 suggests that the number of chromo-

TABLE 2. FREQUENCY OF OTHER CELL CHARACTERISTICS

Chromosome abnormality	Total number observed	Ratio observed/counted *
Breaks	180	.017
Tetraploid cells	119	.011
Endoreduplication	9	.00086
Hyperdiploid cells	32	.0031

* Total cells counted: 10,393.

TABLE 3. RATIO OF TOTAL NUMBER OF BREAKS TO NUMBER OF CELLS COUNTED BY AGE GROUP *

Age	Breaks	Total cells	Ratio breaks/total cells
Females			
0–14	26	1870	.014
15–24	16	783	.020
25–34	13	415	.031
35–44	14	1231	.011
45–64	26	1403	.019
65–74	16	754	.021
75+	7	172	.041
TOTAL	118	6628	.0178
Males			
0–4	1	139	.007
5–14	12	789	.015
15–24	13	642	.020
25–34	16	718	.022
35–44	6	410	.015
45–54	6	437	.014
55–64	7	411	.017
65–74	1	206	.005
75+	0	13	.000
TOTAL	62	3765	.0165

* Similar age groupings as those used in Table 1.

TABLE 4. COMPARISON BETWEEN THE DATA IN TABLE 1 WITH OTHER STUDIES FOR SUBJECTS OVER 65 YEARS OF AGE

	Present report	Jacobs *et al.* (1963)	Hamerton *et al.* (1965)	Jacobs *et al.* (1964)
Males				
Number above 65	5	21	4	87
Per cent hypodiploid *	4.8	8.8	10	7.2
Per cent hyperdiploid *	1.0	2.8	0	0.77
Females				
Number above 65	15	25	7	102
Per cent hypodiploid	9.2	12.8	17.1	12.8
Per cent hyperdiploid	0.95	2.8	2.4	1.4

* Percentages of diploid cells.

some breaks was not influenced by age or sex. Comparisons between age and incidence of other abnormalities for each sex did not show any associations. In addition, there was no obvious relationship between any two of the abnormalities, e.g., subjects with many breaks did not show either an increased or decreased incidence of tetraploidy.

DISCUSSION

Hypodiploidy

The data suggest that hypodiploid percentages in females above 65 years of age were relatively high. A comparison of these data with other reports (Jacobs *et al.*, 1963, 1964; Hamerton *et al.*, 1965) for females over 65 shows some similarities (Table 4). The 15 females above age 65 in our study exhibit hypodiploid percentages of the same order of magnitude as in the other studies. The consistently high incidence of hypodiploid cells in all four studies for the oldest females supports the hypothesis that hypodiploidy is high among these persons. However, there is conflicting evidence that hypodiploidy increased with age in females less than 60 years of age. Our data and those of Hamerton *et al.* (1965) do not show a steady increase in hypodiploidy for females less than 60 years of age.

No evidence of a relationship between age and hypodiploidy in males exists in this study; however, Jacobs *et al.* (1963) and Hamerton *et al.* (1965) reported significant relationships. A summary of the male data from all studies does not give clear, consistent evidence of a steady increase in hypodiploidy with age. An analysis of the appended data in the Court Brown *et al.* (1966) report does not show a relationship between hypodiploidy and age for males.

Hyperdiploidy

The results depicted in Table 1 as well as those of Hamerton *et al.* (1965) show no apparent relationship between age and the percentage hyperdiploidy as does the reanalysis (Court Brown *et al.*, 1966) of the original data of Jacobs *et al.* (1963, 1964).

Degree of Relationship

It is apparent from the scatter of points in Figs. 3 and 4 that a wide range of percentages exists for each age. Such variability was not discernible by the grouped analysis where mean percentages were used for each age group. Consequently the analysis by Jacobs *et al.* (1963) of mean percentages showed a strong relationship. However, an ungrouped analysis of their data (Fig. 4) showed a weak relationship. That is, only about 7% of the variability in hypodiploid percentage is associated with age. This would suggest that other unknown factor(s) are playing an important role in influencing hypodiploid percentage among the specific individuals in the oldest age group who have elevated hypodiploid percentages. From the ungrouped analysis, it appears that female hypoploidy does not increase regularly with age but occurs most frequently beyond age 60. Since there appears to be an abrupt

change in hypodiploid percentage in females at about age 60 (Fig. 1), the factor(s) responsible for the change may be age dependent; however, the responsible factor(s) apparently do not exist among all aged females.

Autoradiographic investigations of an additional selected geriatic population (65 years of age or older) are currently in progress to determine whether the late replicating X chromosome in females is lost more frequently than would be expected by chance alone as suggested by Court Brown *et al.* (1966). Since the observation of increased hypoploidy in older females has been found repeatedly, independent of the sample or selection methods, it may not be necessary to select a probability sample from the general population, which is a difficult and expensive procedure to follow.

SUMMARY

An analysis of the incidence of aneuploidy in 171 subjects (99 females, 72 males) of different ages from the general population was conducted. A least squares analysis based upon individual records showed no significant relationship between age and percentage of hypo- or hyperdiploid cells for either males or females. However, when the data were divided into seven age groups, a high incidence of hypodiploid cells among females above 65 years of age was noted. This finding was in accord with other work and suggests that certain individuals in this age group were different from all the other females. This study of randomly selected individuals shows a wide range of hypo- and hyperdiploid percentages regardless of age and no evidence of a steady increase in aneuploidy between 0 and 65 years of age.

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