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COMPARATIVE STUDIES ON THE ARRANGEMENT OF CHROMOSOMES IN THE C-METAPHASE BETWEEN NORMAL KARYOTYPE AND TRISOMY-21.

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ABSTRACT

Human chromosomes in amnion cells and lymphocytes with normal karyotype and in lymphocytes with pathological karyotype (2n=47,+21) were compared as to their position in the metaphase. None of the collectives showed sex differences.

Measurement of the radial distances revealed more peripheral position of the majority of large chromosomes.

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The satellite-carrying chromosomes of the D group always had a central position in the mitosis.
The chromosomes of the groups D,E,F and G were closest to the centre, with the exception of chromosome 18 which was peripheral in all three collectives.
For the male probands, the y-chromosome was shown in all three collectives to have a smaller radial distance than the x-chromosome.
A typical distribution was found for the radial and homologue distances for the trisomic cells: two of the three chromosomes 21 had a very large radial distance, the three chromosomes 21 had a very large radial distance, the third a value corresponding to its size. For the homologue distances there were two similarly small and one larger measurement whereby the distribution is quite independant of parental source. Comparison of the groups showed no differences either between normal and trisomy cells or between the different cell types.
Examination of chromosomes 6 and 15 proved conclusively that the chromosomes are not particularly orientated in the c-metaphase regarding the position of short and long arm. A preferential combination of particular satellite-carrying chromosomes leads to the frequent fusions of chromosomes 13 and 14, or 14 and 21.
Equally, no preferential association could be demonstrated of the chromosome 21 and the chromosomes with large heterochromatin blocks in the centromere region (Chromosomes 1 and 9). The distances were of the same order of

magnitude as those between chromosome 21 and chromosome 6, a submetacentric chromosome without a marked heterochromatin region. Both latter observations are of specific importance for genetic councelling of couples after birth of a child with a de novo chromosome aberration asking for the recurrence risk.

INTRODUCTION

The present investigation was carried out in order to test whether the additional chromosome in trisomy 21 lymphocytes disturbs the overall arrangement of the other chromosomes. Amnion cells and lymphocytes with normal karyotype were also examined in order to compare the pathological cells with two different, normal cell types.

MATERIALS AND METHODS

For each of the three collectives, 100 metaphases were examined of 50 male and 50 female probands ,and the relative positions of the chromosomes were noted.
Cell division was stimulated by standard culture methods and arrested in mitosis with colcemid. All preparations were stained with quinacrine mustard. All metaphases were photographed and printed at a final enlargement of 2000-2500 X and then measured, six data being recorded for each chromosome. For each measuring point(c=centromere, p=end of short arm, q=end of long arm), x and y coordinates were determined in mm. These data were docu-

mented on a specially developed form, together with the additional data of each proband.

The measuring points, c,p and q of each chromosome were related to the centre of each mitosis. The distance between this point and the measuring point c was designated the radial distance. In addition to the position of the individual chromosomes in the mitosis, the position relative to other heterologous chromosomes was determined, and also the position of its homologous partner. The homologue distance is the distance between the c points of two homologous chromosomes. The position of the sex chromosomes was documented separately. Specific criteria were necessary for the evaluation of the acrocentric chromosomes of the groups D and G (chromosomes 13,14,15,22),which together form the nucleolus in the interphase nucleus (Fig. 1). Careful attention was also paid to the chromosomes 1,9 and 16 since they display especially large areas of heterochromatin in the centromeric regions.

The radial distances and the homologue distances were calculated from the coordinates for each chromosome recorded on the forms. An equation was used, derived from that used by Kirsch-Volders et al. 1977 (17).

For these hypotheses, the programme BMDP-2V (BMDP, P series 1979) was used tp calculate the tail probability of the test quantities presupposing the appropriate null hypothesis. Before the tests were carried out, the maximum probability of a first rate error was set at $\alpha = 5\%$, ie. a result was considered significant if $p < 0.05$.

The Friedman-Test, a nonparametric test of identical position, does not require the assumption of normal distribution for the residual error, but has less power than the analysis of variance. The programme BMDP-3S (BMDP, P series 1979) was used for this test.

RESULTS

1. Amnioncells with normal karyotype

The evaluation of the radial distances for the amnion cells showed a relatively constant standard deviation in the region of 0.5. This observation indicates a generally homogeneous collective and precise definition and measuring procedures.

To begin with, male and female mitoses were documented and evaluated separately. Analysis of variance, however, indicated that there were no differences between male and female probands so that the groups could be pooled.

A mean value for the radial distance of all chromosomes, including the gonosomes, was established at 1.279. In relation to this value, the individual chomosomes could then be described as having a peripheral or central position. Analysis of variance showed a statistically significant difference in the position of the individual chromosomes. The Friedman-Test confirmed this.

The most peripheral chromosome is number 6, which has a radial distance of 1.387. Chromosome 22 is nearest to the centre and has the value 1.105.

All acrocentric satellite-carrying chromosomes have rela-

tively central positions, chromosome 15 being the most central of the D group with a value of 1.168.
The position of chromosome 18 of the E group was conspicuous, being the most peripheral with a value of 1.330 in this group. Considering all the autosomes together, analysis of variance gives the following order from centre to periphery: 22-21-15-20-19-14-13-16-17-10-(X)-11-4-7-3-12-9-18-2-1-5-8-6 The arrangement of the chromosomes begins on the left side with chromosome 22 which has the most central position in the metaphase plate and continues with increasing distance of the different chromosomes to the centre, so that chromosome 6 on the right side of the line has the most peripheral position.
The mean value x is inserted in the sequence.
The order of the chromosomes according to the ranking of the Friedman-Test showed no important differences. If the x and y values are separated for the male probands it can be shown that the x-chromosome, with a value of 1.420, is more peripherally situated than the y-chromosome which has a value of 1.169. A radial distance of 1.331 was given for the female probands (xx).
For the evaluation of the homologue distances, 100 metaphases were analysed, the distance between the homologues of each chromosome measured and a mean for each chromosome pair calculated. There was again a fairly constant standard deviation of 0.8, this demonstrates, that the distances between the single homologue chromosomes are approximately constant.

The homologue distance was usually greater than the radial distance of each chromosome. Analysis of variance showed that there were no sex differences here either. It also showed that the homologue distances were significantly different for the different chromosome pairs. The Friedman Test confirmed this.

The mean value for all chromosomes including the gonosomes, was a homologue distance of 1.757. The chromosomes 6 had a value of 2.041 and were therefore the furthest apart. The closest were the chromosomes 15 with a value of 1.433. This was unusual for the D group, as with the radial distance. Chromosome 18 of the E group again had a considerably larger value (1.907) than the other chromosomes of this group. Beginning with the smallest distance, the chromosomes could be arranged in the following order (according to analysis of variance): 15-19-21-22-20-13-16-X-11-17-14-10-4-12-9-3-1-8-18-5-2-7-6 The Friedman Test gave no important differences.

The arrangement of the chromosomes shows on the left side chromosome 15 with the smallest distance of the homologues and on the right side chromosome 6 with the largest, between them the other autosomes with increasing distances from left to right. These findings on homologues are in good agreement with the observations on radial distances, chromosomes with peripheral position showing the greatest distance of the homologues while those with central position are situated closest together.

For the gonosomes there is a homologue distance of 1.719

for the xy-combination and 1.516 for the xx-combination. The mean distance for the sex chromosomes is therefore less than the mean distance from each other of all chromosomes in the metaphase.

2. Lymphocytes with normal karyotype

The evaluation of the lymphocytes with normal karyotype showed that the values for the radial distances of the chromosomes were scattered about a mean standard deviation of 0.5 which was fairly constant. Analysis of variance here also showed that there were no differences between male and female probands, so that the two groups could be put together. The mean distance of all chromosomes including the gonosomes was 1.276.

Both analysis of variance and the Friedman Test demonstrated a significant difference between the radial distances of the individual chromosomes. Chromosome 11 is the most peripherally situated with a value of 1.367. The most central position was occupied by chromosome 22 with a value of 1.177. Chromosome 1 of the A group was unusual when one considers its size and the positions of chromosome 2 (1.253) and chromosome 3 (1.305). It lies fairly central, having a radial distance of 1.193. The chromosomes of the D group were quite central and had all more or less similar values.

The acrocentric, satellite-carrying chromosomes of group G were also in a central position as well as the chromosomes of group F. The only exception is chromosome 20 in lymphocyte cultures with normal karyotype. In this sample

chromosome 20 had a peripheral position, but the differences as a whole were rather small. The chromosomes could be arranged in the following order, from the smallest to the largest radial distance (with the analysis of variance): 22-1-21-19-15-6-13-2-14-17-7-X-16-12-8-18-9-3-4-10-20-5-11 There were again no important differences with the Friedman Test. For the male probands, a value of 1.351 was obtained for the x-chromosome and 1.312 for the y-chromosome. For the female probands (XX) the radial distance of the x-chromosome had a value of 1.255. As in the amnion cells, the y-chromosome is more central than the x-chromosome. For the homologue distances, a comparison of individual values with the mean showed a relatively constant standard deviation in the region of 0.8. The values for chromosome 20 (0.941) and 21 (0.990) were more scattered. It could again be demonstrated with the analysis of variance, that there are no sex determined differences in the values and that therefore male and female probands could be pooled. A mean value for all chromosomes including gonosomes could be calculated at 1.740. When the homologue distances were tested for significant differences between the values for individual chromosomes, analysis of variance gave a tail probability of 0:5984 and the Friedman Test had a level of significance of 0.7027. This means that there is statistically no difference between the homologue distances. Therefore only positional tendencies can be shown. The chromosomes 5 lie the furthest apart with a value of 1.929. The chromosomes 22

are closest, having a value of 1.607. In all, most autosomes show similar values to those in the amnion cells. Chromosomes 1.6 and 18 differ in that they are closer to each other than in the amnion cells. The differences between the distances are however small and the scatter comparatively large, so that a basically different behavior of the chromosomes in the lymphocytes compared to the amnion cells could not be ascertained. If differences really exist, it might be assumed that the different shapes of the nuclei in the two cell types have an influence on the values observed. The chromosomes could be arranged in the following order of increasing homologue distance (with the analysis of variance): 22-21-17-14-1-19-15-18-6-16-12-x-2-4-8-13-11-3-9-20-10-7-5 . The Friedman Test showed no important differences. The gonosome distances were 1.809 for the male probands (xy) and 1.698 for the females (xx). As for the amnion cells the value of the xx-combination was smaller than that of the xy-combination.

3. Lymphocytes with trisomy 21

For the evaluation of the radial distances for the probands with trisomy 21, the third chromosomes 21 which was selected by chance was initially left out and treated separately later. The other chromosomes showed a fairly constant standard deviation in the region of 0.5. Any differences between male and female probands could again be ruled out using analysis of variance. The mean for all chromosomes including the gonosomes, and excluding chromosome 21, was found to be 1.276. Individual differences

with respect to preferred central or peripheral positions could not be statistically proven with the analysis of variance. The Friedman Test was not carried out since the results up until now had been not different from those of the analysis of variance. The following statements about the radial distances therefore indicate only tendencies for the chromosomes to take up a peripheral or central position. Chromosome 16 is most peripheral, having a value of 1.354. Chromosome 17 is the most central with a value of 1.187. It is striking that in this case both extremes are group E chromosomes. Usually chromosomes of the same group do not differ so much from each other. The chromosomes could be arranged in order from the smallest to largest radial distance (with analysis of variance): 17-3-19-20-2-13-1-9-14-5-22-x-6-15-11-18-8-7-10-4-12-16 For the male probands, the x-chromosome had a radial distance of 1.326 and the y-chromosome a value of 1.281. The x-chromosomes of female probands (xx) had a value of 1.339. As for the cells of normal karyotype, the radial distance of the y-chromosome is smaller than that of the x-chromosome. Analysis of variance also indicated no sex differences with regard to the position of the chromosomes 21. The standard deviation of the individual value from the mean (1.306) was in the region of 0.5. The radial distances of the three chromosomes 21 were as follows: 1st chromosome 21: 1.340, 2nd chromosome 21: 1.199 3rd chromosome 21: 1.380.

Analysis of variance confirms statistically the differen-

ce between the radial distances of the three 21 chromosomes with a tail probability of 0.0372. It seems therefore that two of the chromosome 21 prefer a peripheral position, the third a central, whereby it was impossible to determine from which parent each chromosome came. The homologue distances between the chromosomes 21 are also unusual. The mean distance is 1.713 whereby the standard deviation of individual values scatter more. The deviations were as follows: 0.916 for the distance from the 1st to the 2nd chromosome 21, 0.841 from 1st to 3rd chromosome 21 and 0.792 from 2nd to 3rd chromosome 21. The homologue distances were as follows: 1.391 from 1st to 2nd chromosome 21, 2.107 1st to 3rd chromosome 21 and 1.642 from 2nd to 3rd chromosome 21.

The significant difference of these three distances was statistically confirmed. If the values for radial distances and homologue distances are taken together, the following picture emerges: One chromosome 21 takes up a very central position, the other two lie peripherally and have almost the same radial distance. The two peripheral chromosomes 21 are furthest apart, whereas the distances between them and the central chromosome 21 are almost identically small. Even during evaluation of the mitosis, it was observed that two of the chromosomes 21 were always close together and the third chromosome 21 more isolated. One of the male probands with trisomy 21 had enlarged satellites on one chromosome 21 as a norm variant. It could therefcre be tested whether the non-identical distances

between the three chromosomes 21 involved a particular combination. Examination of 6 mitosis from lymphocytes and 7 from amnion cells showed that this was not the case. The other chromosomes of the trisomy 21 cells demonstrated a fairly constant standard deviation of 0.8 when individual values were compared to the mean.
Differences between male and female probands could also be excluded using the analysis of variance.
The mean of all homologue distances, including gonosomes but excluding the chromosomes 21, was 1.769. A statistically significant difference between the homologue distances could not be shown with analysis of variance. These values again show only trends and are not evident because of the numeric small differences.
Chromosome 9 lay relatively furthest apart with a value of 1.920. The chromosomes pair 3 were relatively closest together at 1.627. The chromosomes could be arranged in the following order of increasing homologue distance(with analysis of variance): 3-17-22-6-20-14-7-15-11-1-x-18-13-19-12-4-16-2-5-10-8-9- For the male probands the xy distance was determined at 2.040 and for the female probands the xx distance was 1.906. Here also, as for the lymphocytes and amnion cells with normal karyotype, the xx distance was less than the xy distance.

4. Comparison of the different cell types
A comparison by analysis of variance of the radial distances in amnion cells and lymphocytes of normal karyotype, showed no differences between the two collectives. The

similar mean values (Amnion cells: 1.279, lymphocytes: 1.276) and the similar order of chromosomes confirms this. In the amnion cells all the chromosomes of the group A,B and C are peripheral to the mean value, in the lymphocytes only the chromosomes 1,2,6 and 7 of these groups have a radial distance less than the mean. The chromosomes of the group D,E,F and G with the exception of chromosome 18, are all more central than the mean in the amnion cells. In the lymphocytes, chromosomes 20 and 16 also have a larger radial distance than the mean, in addition to chromosome 18 which here also has a peripheral position. For both collectives, the x-chromosome of the male probands has a larger radial distance than the y-chromosome. Similar concurrences occur when the homologue distances of the two collectives are compared. Analysis of variance confirms this with a tail probability of 0.3680. The mean homologue distance for all chromosomes was 1.757 for the amnion cells and 1.740 for the lymphocytes. Comparison of the positions of the different chromosomes relative to the mean by analysis of variance and the Friedman Test again demonstrated significant differences in the amnion cells but not in the lymphocytes with normal karyotype. The homologue distances in the group A,B and C, as well as of the chromosomes 14,17 and 18 were larger than the average of all chromosomes in the amnion cells. In the lymphocytes, the chromosomes of groups A,B and C also had values larger than the average with the exception of chromosomes 1,6 and 12. In the groups D,E,F and G the

values were less than mean with the exception of chromosomes 13 and 20. In both collectives, the xy-distance in male probands was larger than the xx-distance in female probands. In the two collectives however the values are different related to the mean distance for all chromosomes: in the amnion cells it was smaller than the mean,in the lymphocytes it was larger but only slightly.
For technical reasons it was not possible to compare all chromosomes simultaneously using analysis of variance when comparing lymphocytes with normal karyotype to lymphocytes with pathological karyotype (2n=47,+21), since it seems pointless to take an average value for the radial distances of the three chromosomes 21 in the trisomy cells. Analysis of variance ruled out any differences between the radial distances of the two collectives when the chromosomes 21 were excluded. The mean radial distance was the same for both collectives and had a value of 1.276. For all further comparisons it must be remembered that for the lymphocytes with normal karyotype a significant difference between the radial distances of the individual chromosomes could be demonstrated, but not for the lymphocytes with pathological karyotype. With a few exceptions, the chromosomes of the normal karyotype lymphocytes could be arranged in order of radial distance so that the larger chromosomes of groups A,B and C were peripheral to the mean, and the smaller chromosomes of groups D,E,F and G were more central to the mean. This was not the case for the trisomy cells, partly since, due to quantitatively

smaller variations, the differences were not so apparent. All the chromosomes of group A were central to the mean as were chromosomes 5 and 9. In addition to chromosomes 18 and 16, which in the normal karyotype lymphocytes also showed larger radial distances than the mean, chromosome 15 had a peripheral position in the trisomy cells too. The values of radial distances for chromosomes 21 were partly peripheral to the mean (x) in the trisomy cells (two chromosomes 21) and partly central of the mean value (one chromosome 21). The latter is closest to the value of chromosome 21 in the normal lymphocyte. An exact identification of the 3 different chromosomes No. 21 was usually not possible because of missing variants.

Basing on our measurements it seems probably that as well paternal as maternal chromosomes 21 take a central or more peripheral position by chance. The collectives were the same with respect to the position of the gonosomes of male probands. In both cases the y-chromosome was nearer to the centre than the x-chromosome. If the homologue distances of the lymphocytes with normal karyotype are compared with those of the lymphocytes with pathological karyotype (2n=47, +21) using analysis of variance, a difference between the two collectives can be ruled out; chromosome 21 being left out for technical reasons. As for the radial distances, there did not seem to be any point in taking an average value of the three possible homologue distances for the chromosomes 21 of the trisomy cells, since such a value would cancel out any possible

differences. The mean of all chromosomes was 1.740 for the lymphocytes with normal karyotype and 1.769 for the lymphocytes with pathological karyotype (2n=47, +21), when chromosome 21 was excluded. When comparing the two collectives it must be remembered that for neither group could a significant difference for the homologue distances of the chromosomes be proven. The homologue distances of the groups A,B and C with the exception of chromosomes 1,6 and 12 were larger than the mean in the lymphocytes with normal karyotype. The chromosomes of groups D,E,F and G all had homologue distances less than the mean with the exception of the chromosomes 13 and 20.

The trisomic cells showed more exceptions. The chromosomes 1,3,6,7 and 11 of the groups A,B and C had smaller values than the mean. In the groups D,E,F, and G chromosomes 13,16,18 and 19 are the exception in that they have larger values for homologue distances than the mean.

The homologue distances between the three chromosomes 21 in the trisomy cells were very remarkable. The distance between the 1st and 3rd was definitively above the mean at 2.107, whereas the distances between the 1st and 2nd (1.391) and between the 2nd and 3rd chromosome (1.642) were below the mean and therefore nearer to the value in normal karyotype cells which was 1.630.

Both collectives show results in agreement with those for the amnion cells in that the distance between the sex chromosomes for the male probands (xy) is larger than that for the female probands (xx).

5. Particular orientation of the chromosome in the mitosis

For all observations reported above, the centromere of a chromosome(measuring point c) has been regarded as the single relevant point. However it is possible to test whether a chromosome preferentially takes up a particular orientation in the c-metaphase, ie. whether the long or short arm of a chromosome has a favoured attitude to the centre of the mitosis.

Chromosome 6 seemed particularly suited for testing this question as it is large and usually lies peripherally. The radial distances for the amnion cells were as follows: p 1.389, c 1.387 and q 1.424. The standard deviations were in the region of 0,5 for all three measuring points. A statistical comparison using analysis of variance showed no significant difference between the points c,p and q. Chromosome 6 therefore has no particular orientation in the mitosis. Chromosome 15 was also examined in this way. It is an acrocentric chromosome in group D and has the smallest radial distance in the amnion cells. The radial distances of the three measuring points were as follows: p 1.172, c 1.168 and q 1.193. The standard deviations were in the region of 0.5. A statistical comparison using analysis of variance showed no significant difference between the measuring points c,p and q. It can therefore be said that chromosome 15 also does not have a particularly favoured orientation. Since neither for the extremely peripheral submetacentric chromosome 6, nor for the centrally placed acrocentric chromosome 15 could an orienta-

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tion within the mitosis be demonstrated, an alignment of the human chromosomes in the c-metaphase seems unlikely.

6. Associations of satellite carrying chromosomes.

The investigation of the distances between single, non-homologous chromosomes in group D and G in amnion cells, lymphocytes with normal karyotype and lymphocytes with pathological karyotype (2n=47,+21), was based on the well known facts that of 53 centric fusions between chromosomes D and G in man, 46 are a 14/21 combination (Mikkelsen 1971), and that the centric fusion 13/14 is the most common of all structural aberrations. If the chromosomes 14/21 and 13/14 are more strongly associated in the interphase nucleus than the other chromosome combinations, then this would be a possible explanation for the frequency of these chromosome coalescences.

Of the 4 possible distances between 2 heterologous chromosomes or their homologous partners (Fig. 1), for the chromosomes 21 of the trisomy cells there were 6 possible distances, the extreme values were taken for each mitosis. There was therfore a largest possible and a smallest possible distance between chromosomes. There was calculated an average of these values from the 100 probands. A projection of these values was necessary so that the inconsistent values could also be documented since there could be simultaneously in any mitosis, small distances between heterologous chromosomes or between their homologous partners which could lead to an association, and also a relatively large distance to the second heterologous chromo-

some which would make an association impossible. If only the mean of these 4 distances were considered, all the values would be evened out and a tendency to associate would no longer be recognisable.

As a comparison, a mean was calculated of the 4 distances, for the chromosomes 21 of the trisomy cells it were 6 distances, and the average then found for the 100 probands of a collective. This gave the mean distance. The standard deviations were about 0,5 and fairly stable with in each group. The distances between chromosomes 13/14, 13/15 and 14/15 were also investigated. In none of the groups could particularly small or particularly large distances be demonstrated, and between the collectives there were no unusual differences between the values. The distances between chromosomes 21/13,21/14 and 21/15 were in all three collectives approximately the same as the distances between the D chromosomes. Here also, no particular associations could be shown.

The distances between chromosomes 21 and 22 were, as exspected, the smallest of the minimal distances and the smallest of the mean distances for each of the three collectives investigated.

In all, therefore, the heterologue distances could not provide a reason for the increased tendency of chromosomes 21/14 and 13/14 to fuse with each other.

7. Position of chromosome 21 to chromosomes with large centromeric heterochromatin blocks.

Large familial investigations and analysis of parents

having children with a free trisomy 21, have led to the suggestion that the especially large heterochromatin blocks in the centromere region of chromosomes 1 and 9 might possibly effect an association with chromosome 21 causing more frequent nondisjunction at metaphase 1 of meiosis (Ford 1978). Should this be case, it would be expected that the centromeric distances 1/21 and 9/21 would be smaller than, for example, the distance between chromosome 21 and chromosome 6, which possesses a very small centromeric heterochromatin block. Here also, the minimum, mean and maximum distances between heterologous chromosomes were ascertained.

On the basis of these measurements, it could be shown in all three collectives that there is no reason to suppose an attraction of chromosome 21 to the large heterochromatin blocks at the centromere.

DISCUSSION

Previous publications on the position of the chromosomes in the metaphase, that have dealth with fibroblast cultures have, with a few exceptions, mainly examined lymphocyte cultures (16,31). In general, it can be deduced from most of the papers that the large chromosomes lie mainly on the periphery of the mitosis, while the small chromosomes stay more in the middle (17,3,27,12, 15). Occasionally, an independance of chromosome position and size is also described (3).

The results of this investigation showed that the chromo-

some positions in the metaphase of amnion cells and lymphocytes are not basically different. In both groups, the large chromosomes were more peripheral and the small chromosomes were more central.

In particular, the values for the radial distance were found to be similar to those of KIRSCH-VOLDERS (17). The chromosomes of groups D and G were always decidedly more central than the mean, the B-chromosomes more peripheral to the mean. The previously described, too large radial distance of chromosome 18, considering its size (32) could also be confirmed by this investigation. The homologue distance also appears relatively too large for chromosome 18, whereby this was more obvious in the amnion cells than in the lymphocytes.

In the lymphocytes, chromosome 1 had a too small homologue distance for its size. This value could be due to the postulated attraction of the heterochromatin blocks of homologue chromosomes (31,3,18), although from our findings this would seem unlikely (30) since chromosome 9, which possesses much more extensive heterochromatin blocks relative to its size (25-35% of the total length as opposed to 10-20% of the total length of chromosome 1; 28), showed no such homologue attraction.

There was not much information available for a comparison of homologue distances. The results of this work showed that the homologue distances were occasionally smaller than the distances between heterologues chromosomes, but that no general rules could be deduced from this. HENS et

al. (14) examined the homologue distances for the acrocentric chromosomes. As in the present study, the measurements were among the smallest, chromosome 15 being an exception. Whereas this chromosome was found by HENS et al. to have the largest value, we found the homologue distance in the amnion cells to be the smallest of all chromosomes, and in the lymphocyte cells it was also very small. In none of the three types of cells investigated could any indication be found that the position of the autosomes were different in male and female probands, either for the radial distances (in agreement with KIRSCH-VOLDERS et al., 17) or for the homologue distances.

With respect to the gonosomes, it could be shown that in all three collectives, the y-chromosome had a smaller radial distance than the x-chromosome. This statement is supported by the work of MORISHIMA et al. 1964, GRUMBACH et al. 1963, MURKHERJEE et al. 1964 and OCKEY 1969 (17). HAGER et al. (12) also determined a more central position for the y-chromosome and a more peripheral position for the x-chromosome when investigating the translocation in lymphocyte cultures after Trenimon treatment.

The three collectives also showed similar results for the homologue distance of the gonosomes. The xy distance in male mitosis was always larger than the distance between the two x-chromosomes in female mitosis.

If one assumes that the second inactive heteropycnotic x-chromosome (Barr body) takes up a peripheral position (16,4) then consequently the other x-chromosome must al-

ways be central. Another possibility is that the heteropycnotic x-chromosome changes its position in the metaphase, however there is no proof of this happening. Only in meiosis could such a small distance between the two x-chromosomes be postulated so that even crossing over would be possible (6,7).

A comparison of the radial distances and the homologue distances in the lymphocytes with normal karyotype and with trisomy 21 indicated no basic differences in position. The additional chromosome 21 does not disturb the overall structure of the mitosis.

Divergent from these findings HENS observed an alteration of chromosome arrangement in trisomic cells of chinese hamster fibroblasts compared to cells with normal karyotype (13).

The position of the three 21 chromosomes were unusual. Two of them had a relatively large radial distance, the third corresponded to the value for chromosome 21 in normal karyotype mitosis.

For the homologue distances there were two smaller, similar values, between the central chromosome 21 and the two more peripheral ones, and one larger distance between the two outer chromosomes.

As far as the tendency of acrocentric chromosomes generally to associate is concerned, the trisomy cells were not different from the cells with normal karyotype. ZANKL and NAGL (33) also reached this conclusion in the investigation of a trisomy 21 mosaic. The parents of trisomy

21 children also showed no increased tendency of the chromosomes 21 or other acrocentric chromosomes to associate (20). Acrocentric chromosomes often fuse at the centromeres with the loss of the short arms and usually one of the centromeric regions (4). Carriers of such fusions possess only 45 chromosomes. Of all acrocentric chromosomes numbers 13 and 14 or 14 and 21 are most frequently fused together (22). This study however, indicates that particular tendencies to associate or a preferential positioning of the chromosome in mitosis as a precondition for this type of fusion, is unlikely. The results for both amnion cells and lymphocytes with normal karyotype indicate no preferential coming together of particular groups of acrocentric chromosomes.

There was therefor no increased frequency of association of homologous acrocentric chromosomes compared to the association of heterologous acrocentric chromosomes as describes by GURBANOV (11). In addition, a sex difference with respect to the frequency of satellite association (19) in the three collectives could not be demonstrated. Differences in the position of individual non-acrocentric chromosomes (such as chromosome 1 and 6) in the different cell types can probably be explained by the different shaped nuclei. Lymphocytes have spherical nuclei, amnion cells are more flat and oval. This would explain that chromosomes that have a fairly large distance from the centre of the mitosis in a spherical nucleus, might well show a fairly small distance from the centre after the

nucleus has been made to burst on a slide and the chromosomes are all on a two dimensional plane.

All the mitosis in this investigation were treated with colcemid. The influence of colcemid on satellite association is not altogether clear. Most authors dismiss any effect (9,2,25,5,29,1,30). A few authors however, consider colcemid as the most important factor that can affect the position of the chromosomes (3).

Hypotonic pretreatment of human and plant cells is also regarded by the majority of the investigators as having no influence on the position of the chromosomes in metaphase (18).

All in all, our own examination of amnion cells and lymphocytes, as well as the majority of the above publications on this theme, show that the arrangement of chromosomes found is not an artefact determined by colcemid or hypotonic treatment, but rather that the values found mirror the natural relative positions of the chromosomes.

In summary it can be said that the present study could, because of the large number of probands, demonstrate a generally valid new understanding of the positions of the chromosomes in different cell types. In some cases existing results could be confirmed, other statements could be repudiated with great certainty.

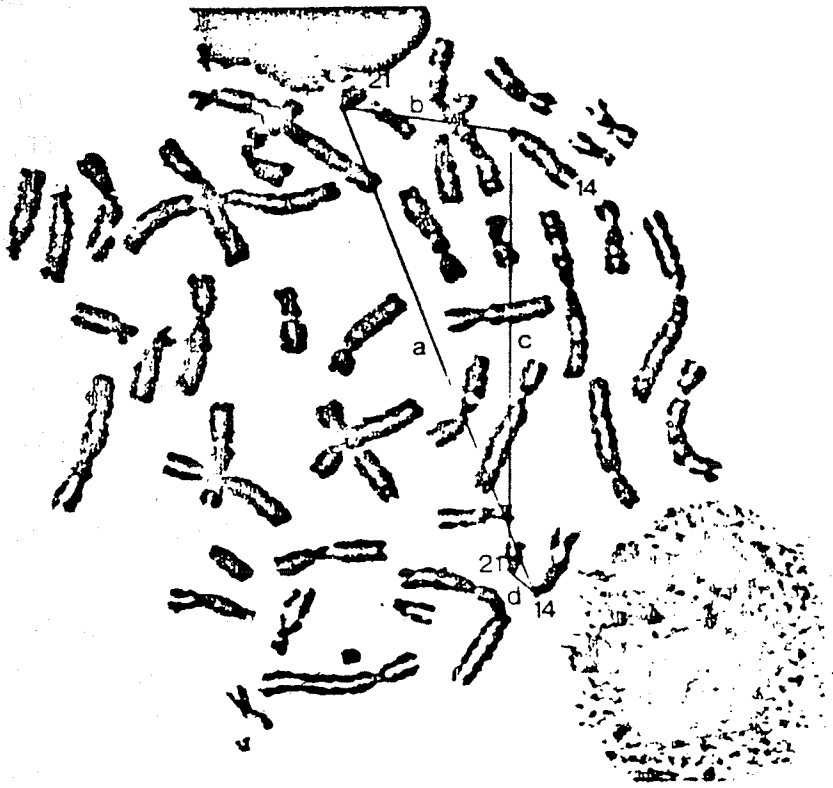

Fig. 1: Description of the 4 possible distances between the chromosomes 14 and 21 (chromosomes stained in Giemsa-Banding-technique).

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