Somatic Chromosome Complex of the Human Embryo

By

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I. Introduction

Investigators of the chromosome complex in man, through work with testes (ovaries being difficult to obtain we have as yet no satisfactory karyological data on oogenesis in man), have come in the main to a uniform conclusion. Putting aside the data which, owing to unsatisfactory techniques are at present only of historic interest, it may be pointed out that for a number of years discussion was centered on the presence or absence of the Y-chromosome in the heterogametic sex. The works of Painter (1921–23), Evans and Swezy (1929), Andres and Jivago (1932), Andres (1933), Minouchi and Ohta (1932) have indicated that the diploid number of chromosomes in man (in both sexes) is 48. This assumption is based not only on direct cytological investigation of orthoploid sets but is also confirmed by the existence of a polyploid or diploid set in the spermatogenesis of man (Andres, 1933).

Different results, however, have been obtained by those who have studied the somatic tissues of the embryo in sections, in total membranes, or in tissue cultures in vitro.

In spite of the fact that theoretically we might expect results identical to those obtained in gonads, the actual data do not correspond to these assumptions and present a considerably varied picture.

Recent studies may be summarized as follows:

1. There is no constancy in the number and morphology of chromosomes in the somatic cells of the embryo.

   This is the conclusion of Karplus (1929). He studied the total serous membranes from one embryo 123 mm in length and the amnional membranes of four foetuses 21.5 to 40 mm long. The number of chromosomes varied from 30 to 64 in separate cells.

2. There is no definite somatic number. It varies within certain limits.

   This is the conclusion of Grosser (1921–27). Considering data from several amnions, he gives the number of chromosomes as 30
to 50. Rappeport (1922) in her material on total serous membranes of the pleura and amnion, observed in eleven counts 40 to 44 chromosomes, the most probable number being 40 to 42. Caffier (1932) frequently observed 45 to 50 chromosomes (in 12 out of 18 counts) in total cultures in vitro of the lung of an embryo. Less frequently he observed lower or higher numbers varying from 30 to 85.

3. There is a definite number of somatic chromosomes, not 48 but 24 or an approximate number.

Here belongs the work of ShakhoV (1926–27) and Adamstone (1932). ShakhoV worked out 30 cases. The material consisted of sections of chorion and decidal tissue. Out of 107 counts performed by the author 84 gave 24 chromosomes, 16 gave 48 (the author considers them as split, but not yet divided, daughter cells), 3 gave 23, 3 gave 18–20 and one gave 8. Adamstone studied one embryo 8.5 mm long. As is evident from his short communication he obtained 24 or an approximate number.

4. The somatic chromosome complex in an embryo is identical with the chromosome complex of the gonads.

This is the conclusion of Evans and Swezy who carried out 46 counts on two embryos, one male 19.5–20 cm long (17 counts), and one female 25 cm long (29 counts). A less categorical statement is made by Kemp who studied tissue cultures in vitro from three males and one female embryos and who selected for his counts 25 figures out of thousands of mitoses observed by him. The former authors reported the number of chromosomes as 48 for both sexes and the latter reported 47 to 50, with the most probable number as 48 and with 1 or 2 chromosomes in some counts as doubtful.

Painter, 1930, in summarizing his study on the chromosome complex in man, tried to explain the results obtained by Karplus by the fact that the latter had to deal with pathological material. From the data given above, it is evident that his results are not at all singular.

Painter’s considerations undoubtedly may be significant but they are not sufficient to completely explain the deviation from the diploid number observed by some authors in the soma of an embryo. On the other hand, taking into consideration the variety of the results and the limited material obtained by each author, the variation in the number of chromosomes becomes of primary importance, especially since similar data have been obtained in pigs (Sus scrofa) by Hance, in Amphibia by Della Valle and especially in birds by several authors. P. I. Jivago suggested, as an interpretation of the variations in the somatic chromosome counts, that these variations
constituted phases of the regular development of the karyotype during ontogenesis. He developed this point of view in a paper delivered at the First Conference of Histologists in Moscow (1934) and in a number of other publications.

Cases of polyploidy of certain tissues in insects are known (Wilson 1928) in which, apparently, histogenetic factors are involved. Thus, S. Frolova has established the frequency of polyploid cells in the rectal glands and trachea of some diptera. Still more complex cases were observed by Sanderson in Pteromidea ribesii scop. The females of the insect with the diploid number 16, had 16 chromosomes in cells of the connective tissue and blood, 32 in the hypodermal tissue, and about 120 in the fat cells (these cells are nearly 16-ploid). The males which developed from unfertilized eggs were haploids, and had 8 chromosomes in the connective tissue, and 16 in the hypodermal tissue. The fat cells of the males had a still greater number of chromosomes but the author could not establish the exact number. Therefore it is possible that similar phenomena may take place in the histogenetis of other organisms.

Particularly in man, Hansemann has already pointed out a difference in the number of chromosomes in cells of various tissues. The number of chromosomes in the cells of the epidermis is, according to him, higher than in the endothelium of the vessels. These considerations led us to further investigate the problem of the somatic chromosome complex in the embryo of man on a large scale and with systematized material. The aim of our work was, first, to verify the presence or absence of variations in the number of somatic chromosomes in the human embryo, employing normal material and as far as possible covering different periods of embryo-genesis; secondly, in case of chromosome variations, to find out their possible relations to definite histogenetic differentiations; and thirdly, to make an attempt to elucidate the development of these variations.

II. Material and Methods

In order to investigate, karyologically, various definite tissues of the embryo we chose the following specimens:

1. Extra-embryonic ectoderm and mesoderm. We used the amnion for the former and the parenchym of the villus of the chorion for the latter, as did a number of previous authors.

2. In the foetus we limited ourselves to the study of the skin (frontal, abdominal wall, hip and face), brain, lung and small intestine.
From the derivatives of the outer layer we obtained the skin epithelium, from the medial layer we obtained mesenchyme of the skin, intestine and lung, from the derivatives of the inner layer we obtained the epithelium of the lung and intestine and from the derivatives of the nervous plate we obtained the brain.

A comparative study of extra-embryonic tissue and tissues of the foetus is of interest. Some authors (Winiwarter and Oguma 1926, Darlington 1932, Kemp 1930) consider the membranes of the foetus as "tissues of a special kind" (Kemp) or "tissues ephemères" (Winiwarter and Oguma) and do not generalize from data obtained on such material.

The material we dealt with consisted of 36 embryos, 3 amnions and 1 scrape of endometrium containing pieces of the membranes of the foetus (chorion and decidual tissues). 19 embryos were abortions. The age of these embryos was from 6 to 10 weeks. Their sex could not be identified. Seventeen embryos were obtained by different operations per laparotomiam. The total length of the embryos was from 5.5 to 32 cm (a total length corresponding to an age of from 75 days to 5 1/2 months). They were of both sexes. Three amnions were obtained from foetuses aged 6, 8 and 10 weeks. One was male, another female, and the sex of the third we could not determine. Sex was established macroscopically. We wish to thank Prof. W. Bunak for the help in the identification of sex of the embryos. The decidual tissue and chorion were obtained from a 3–4 weeks old pregnancy. Thus in our material we had an age range of 3 weeks to 5 1/2 months.

The material was obtained in the following manner. Scrapes extracted by curette spoon from the uterus were immediately sliced and placed in the fixative. We took only those pieces extirpated at the very beginning of the operation. Thus the period from the destruction of the foetus till the moment of fixation did not exceed one minute.

The embryos obtained by laparotomia were treated as follows: after opening the uterus, the foetus covered with all the membranes was handed to us directly from the operating table. After the opening of the chorion it was placed, with the amnion, in a fixative (in this way we used only the amnion). In some cases the embryo was opened, the necessary tissues and organs were extracted, sliced, and immediately placed in the fixative. All these operations, from extirpation of the embryo from the uterus till fixation never lasted over a minute.

For supplying the materials and for personal help in their fixa-
tion we desire to thank Prof. J. Vogel director of the Gynecological Clinic of the 2nd Medical Institute in Moscow.

Samples of endometrium containing pieces of chorion and decidual tissue were placed directly after extirpation from the uterus in the fixative where they were sliced with scissors. The whole process of extirpation, fixation and slicing hardly lasted 15 seconds.

For fixation of tissues and pieces we used the method of Painter. The tissue was placed for 6–8 hours in a strong solution of Flemming fluid and then for 16–18 hours in Herman fluid. A part of the material was fixed with Flemming fluid. Chloroform and paraffin were used for imbedding according to routine histological technique. The amnion was fixed with Flemming solution and only the total membranes were studied. The use of serous membranes obtained by Rappeport's method (slicing by forceps) was avoided, partly because this operation takes more time and partly because the tissues are easily damaged.

The material imbedded in paraffin were studied serially. The thickness of the sections was 8 microns. The sections were stained with Toluidin-blue according to Michaelis with Safranin or Acid Fuchs in as counterstain; iron haematoxylin was used according to Heidenhain and the Feulgen reaction was used. In the latter for better contrast with the plasm, we used water-blue as a counterstain according to the method described elsewhere (Jivago and Andres).

All drawings were made at the desk with Reichert homogeneous 1/18 oil immersion objective and Reichert 25× eyepiece. The details were drawn with Reichert binocular of 12×. Photomicrographs were taken with the same objective and a 12× eyepiece, and they were enlarged in reproduction.

III. Characterisation of Material

Mitoses were observed in all sections but with varying frequencies. As has been noted by all investigators the number of mitoses decreases with age. The organs of the embryos above four months old revealed only occasional cell divisions. The intensities of cell division of different tissues are apparently unequal and each of them evidently has its specific periods of minimum and maximum. Data concerning human material are sometimes encountered in the literature. Thus Fishel points out that the maximum of mitoses in the epithelium of the intestine takes place during the second month of embryogenesis, after which it sharply decreases. But besides organogenesis we have to consider the particular rate of cell division of the embryo in question. Thus, for instance, in the abortion material of almost the same age we observed a different number of mitoses
even in analogous tissues. The main data for our counts were supplied by embryos 5.5 and 7 cm long (2.5 and 3 months old) and partly by an embryo 17.5 cm long (4 months old). The sex of the first could not be determined, the other two were male embryos. A considerable number of counts were carried out on the amnion of a female embryo 8 weeks old.

Concerning frequency of different phases, we may note that, as is usually found, the most numerous were the prophases and the least numerous were typical metaphases, a fact which is a natural expression of the relative duration of the corresponding phases.

The great majority of mitotic figures were of a normal bipolar mitosis, though we constantly met, in addition, a certain number of polypolar and aberrant forms, and also asymmetries with intruding isolated chromosomes or even whole groups of chromosomes. This question will be dealt with later.

In the morphology of mitoses we must first note the different sizes of the mitotic figures in the cells of various tissues. This is partly related to the sizes of corresponding cells, but this is apparently not the sole cause of this phenomenon.

The mitoses in cells of the epithelium, skin, and lung are of small size, those in cells of the connective tissue are somewhat larger. The mitoses in some cells of the amnion are considerably larger. The form of the chromosomes in different tissues has also its peculiarities. In successfully fixed and stained sections the chromosomes of the epithelium appear thinner, though shorter than the chromosomes in the cells of the connective tissues. In the amnion we can clearly distinguish two different types of mitotic figures: in the larger polygonal cells, the chromosomes during the metaphases are thin, slender, and comparatively long, while in cubical or flat cylindrical cells of smaller size the whole figure is smaller and the chromosomes are smaller and thicker.

In the analysis of chromosomes we considered it necessary to investigate a sufficient number of prophases and metaphases for each kind of tissue. We did not fully succeed in the case of metaphases. We attempted to use complete figures situated in one section, though in some cases, on account of the clustering of chromosomes, it seemed better to use figures situated in two sections. It was obviously necessary to pay great attention to the serial studies of our material. For our investigation we selected macroscopically and microscopically normal material. With a no less severe criterion we considered each cell. We paid great attention to an absence of local changes in tissues which might be considered as pathological, such as local haemorrhages and degeneration. All more or less suspicious cases were excluded.
We did not make any counts of polypolar mitoses and limited ourselves to an analysis of bipolar mitoses.

Taking into consideration that some of the chromosomes could be cut in two and thus counted twice and that in view of technical difficulties one or two chromosomes could not be properly classed, we divided the obtained data into the following groups:

I. **Subdiploid**, including all cells from sections with number of chromosomes up to 45, and up to 46 from the amnion, whose cells are larger and figures are easier to count.

II. **Orthoploid**, including cells from sections with number of chromosomes from 45 to 51, and from 47 to 49 for the amnion.

III. **Hyperploid**, includes all cells with number of chromosomes above these limits.

Thus the orthoploid group consists of all cells having variations in the chromosome counts of 3 above or below the diploid number (48) and of the amnion having a variation of 1.

The results of our counts are tabulated below:

<table>
<thead>
<tr>
<th></th>
<th>Prophases</th>
<th>Metaphases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>A. Extra-embryonic tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Ectoderm (amnion)</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>b) Mesoderm</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>B. Embryonic tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Ectoderm:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Skin epithelium</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>b) Mesoderm:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connective tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. of the skin</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>2. of the intestine</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3. of the lung</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>c) Endoderm: Epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. of the intestine</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2. of the lung</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>d) Epithelium of the nervous tube:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. brain</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>51</td>
</tr>
</tbody>
</table>

Note: This number is below the actual one, as we selected in this case only low chromosome numbers
As is seen from the table, out of 159 mitotic figures some 49% belong to the orthoploid group, the remaining 81 cells are divided between the subploid (I) group which includes 35 cells (22%) and hyperdiploid group (III) which includes 46 cells (29%) (Text-figs. 1-18; Pl. 15, Figs. 1-7).


We could not establish any essential difference between various regions of the embryonic soma in the karyological characteristics based on our material. Everywhere the cells of the orthoploid group are the most numerous, but at the same time we met variations towards hypoploids and hyperploids. It should be pointed out that marked variations from the diploid number are seldom met, whereas the maximum and minimum counts are rarely met; the limits of variation (32 to 73 chromosomes) are comparatively wide.

The counts of all three groups are equally represented in nuclei lying in one or two sections. In the section of the brain we
observed 51 chromosomes in a case of reconstruction of the nucleus from two sections. In the drawing of one of the other sections we observed 73 chromosomes, the highest number in our material (Text-fig. 9, Pl. 15, Fig. 2 a, b, Fig. 3 a, b, c, d, e). The variations are far from representing a correct polyploid range and though we meet multiple haploid numbers (48; 72) or numbers approximating these, the majority of the elements are heteroploids. A summarized comparison of prophases and metaphases shows that the ratio of the orthoploid and other groups is almost equal (47.2% and 52.9%). But comparing embryonic and extra-embryonic tissues in each phase, we notice a considerable difference in the frequency of hypo- and hyperploid cells. In the prophases of the embryonic tissue (in the

Text-figs. 7-12. 7. Nucleus of the cell from the villus of the chorion. The same as in Plate 15, Fig. 1. 38 chromosomes. 8. Another nucleus from a similar cell, from two sections. 71 chromosomes. 9. Nucleus from a brain cell, the same as in Plate 15, Fig. 2. 73 chromosomes. 10. Nucleus from another brain cell, as represented in Plate 15, Fig. 3. a corresponds to a, b, c in photomicrograph 3; b corresponds to d and e in photomicrograph 3. 11. Skin epithelium. Metaphase. PAINTER-MICHAELIS. 47 chromosomes. 12. Nucleus from a histiocyte of the skin, as in Plate 15, Fig. 4. b is from another section of the same cell. 32 chromosomes.
derivative of all three layers) the hyperploid group is comparatively numerous and the hypoploid small, whereas among the prophase of the cells of the extra-embryonic tissue the reverse is true, that is, the hypoploids are well represented. In the metaphases of both the embryonic and extra-embryonic tissues the hypoploid group prevails. We cannot explain the prevalence of hyperploids in the prophase of the embryonic tissue as due to a defect of our material. Higher numbers in the set of chromosomes during prophase have also been noticed by other observers (Popoff—in amnion of a chicken, and Hance in embryo of a pig). Hance noticed the presence of higher numbers in the prophase as compared with metaphases. He speaks of a temporary fragmentation and considers that later fragments may combine into entire chromosomes again. He emphasizes the fact that the total length of chromosomes in various prophase and metaphase nuclei are almost equal, independent of the number of chromosomes.

Text-figs. 13-18. 13. Nucleus from a histiocyte of the skin, as in Plate 15, Fig. 6. 54 chromosomes. 14. Nucleus from another histiocyte of the skin, as in Plate 15, Fig. 7. 50 chromosomes. 15. Nucleus from a third histiocyte of the skin. Prophase. Painter-Feulgen. a = one, b = second section. 56 chromosomes. 16. Histiocyte of the small intestine. Metaphase. Painter-Feulgen. 57 chromosomes. 17. Histiocyte of the small intestine. Metaphase. Painter-Feulgen. 49 chromosomes. 18. Nucleus from one epithelial cell of the small intestine, drawn from two sections (a and b). 45 chromosomes.
But the proofs of secondary reconstruction of chromosomes are doubtful. We are inclined to think that probably in the prophase of the chromosomes occurs which leads to an apparent increase in their number. Whether or not we must consider this as a specific karyological characteristic of histological determination is still an open question.

We must treat separately the number of orthoploids in the metaphases of the amnion. As is seen from the table, this percentage is lower than in the metaphases of the embryonic tissues. We consider that the number obtained is lower than is actually so, since, in view of our special considerations, we have mainly chosen for our counts figures with few chromosomes.

IV. The Mechanism of Formation of Heteroploids in the Embryonic Soma

The following factors may be considered responsible for the deviation from the diploid number of chromosomes in the mitoses:

1. Polypolar and monocentric mitoses
2. Fragmentation of chromosomes
3. Association of chromosomes
4. Non-disjunction of one or several pairs; with a great number of pairs this gives an asymmetric mitosis
5. Elimination of chromosomes
6. ROSENBERG's restitution of the nucleus
7. Synkaryogamia
8. Somatic reduction.

Fragmentation and restitution lead to an increase in the number of chromosomes, while association, elimination, synkaryogamia and somatic reduction lead to a decrease. Polypolar mitoses, non-disjunction (asymmetric mitoses) lead to an increase in number in some and to a decrease in other cells.

Neither restitution nor synkaryogamia play any marked rôle in the formation of variations of the chromosome complex. This is evident from the fact that we meet variations on both sides of the diploid number, and from the fact that there is absence of high degrees of polyploidy, whereas we might expect variations only above the polyploid number. The maximum sets observed were close to triploids (from 65 to 73 chromosomes) but we met only 5 cells of this kind in our material.

In the same way we must exclude the active rôle of somatic reduction, the possibility of which has been recently pointed out by PAINTER, as we did not observe numbers below 32, and among the more frequent, not less than 37. Neither can a significant rôle be
played by elimination which acts only in the direction of a decrease of chromosome number. Fragmentation, to which a considerable rôle in the evolution of the chromosome sets has been attributed (DARLINGTON, REUTER, BELIAJEW) and which, together with the association of fragments, plays an important rôle in the qualitative reconstruction in separate chromosomes inside the set, cannot be considered as a leading factor in the variation of chromosome number, because the number of kinetic bodies which determine the existence of individual chromosomes remains unchanged. A result of fragmentation will be that the fragments, being loose from the spindle attachment and not being regenerated, will be eliminated during the next diakinesis. DARLINGTON allows a possible regeneration if the fragments are sufficiently large. But there is not yet sufficient proof of this possibility (NAVASHIN 1929). It is somewhat difficult to determine the rôle played by the association of entire chromosomes. Cases are known of a stable existence of complex chromosomes formed in this way (the linking of the X-chromosome in Drosophila). But there is considerable evidence in the literature against the assumption that this mechanism has wide significance in the change of the number of chromosomes in the set (M. NAVASHIN 1932). Our material confirms this negative point of view, as we have both hypoploids and hyperploids.

Paying special attention to the spindle fiber attachments of individual chromosomes, we failed to find any morphological indications (double twisted chromosomes etc.) that there can be more than one spindle fiber fixed to a chromosome. Our searches proved also unsuccessful in the amnion treated according to FEULGEN, where we met several mitotic figures with separate chromatised traction spindle fibers, clearly differentiated from those of the mantle fibers, which remained unstained.

There are many indications in the literature of the rôle of polypolar and partly asymmetric mitoses as factors causing deviations from the diploid number. Within the limits of the embryonic soma in man, KARPLUS and CAFFIER attribute great significance to these factors. Those authors have not given very serious consideration to the relative rôle of separate kinds of aberration in the process of mitosis which cause the development of heteroploidy.

We approached this question statistically. Considering that elimination and non-disjunction give mostly retardation in several pairs in the fixed material, that non-disjunction in many pairs gives asymmetric mitoses, and that the polypolar mitoses are closely distinguished, we counted all the mitoses encountered and classified them accordingly. We must stress that it is impossible to consider
all cases of retardation as an expression of elimination or non-disjunction. From the observations of Belar we know that a “retained” chromosome may “overrun” the others at the end of mitosis. Nevertheless in the majority of cases the interpretation of “retardation” in the sense mentioned above will correspond to reality. It is interesting to note that some authors mention a “mirror image” position of the retained pairs which shows that a definite connection exists between them (Caffier). The results obtained in our counts are tabulated below:

Table 2. Distribution of mitosis according to type

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of investigated mitoses</th>
<th>Among them</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Asym-</td>
<td>Retained</td>
<td>Multi-</td>
<td>Aberrant %</td>
</tr>
<tr>
<td>Amnion</td>
<td>300</td>
<td>278</td>
<td>1</td>
<td>19</td>
<td>2</td>
<td>7.3%</td>
</tr>
<tr>
<td>Skin (connective tissue)</td>
<td>100</td>
<td>93</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>7.0%</td>
</tr>
<tr>
<td>Intestine (epithelium and</td>
<td>500</td>
<td>471</td>
<td>2</td>
<td>22</td>
<td>5</td>
<td>6.3%</td>
</tr>
<tr>
<td>connective tissue)</td>
<td></td>
<td></td>
<td></td>
<td>46</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>900</td>
<td>842</td>
<td>4</td>
<td>46</td>
<td>8</td>
<td>6.4%</td>
</tr>
</tbody>
</table>

As is seen in this table, in all embryonic tissues there is an almost equal percentage of mitoses causing an abnormal distribution of chromosomes. But this percentage is much lower than one might expect from a direct count of the relation between the orthoploid and heteroploid sets (6.4% of aberrant mitoses and about 50% of heteroploids). This discrepancy becomes clear if we consider that in our classification of all phases, prophase (the majority) and metaphases of bipolar mitoses fall in the normal group, because it is impossible to foresee from them the distribution of chromosomes among the daughter cells in the future. Therefore only a selective analysis of anaphases and telophases can give a more exact picture of the frequency of the abnormalities in the dynamics of mitosis. These data are tabulated below:

Table 3. Frequency of disturbance in mitosis (anaphase and telophase)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of investigated mitoses</th>
<th>Among them</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Among them % of retained chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal Asym-</td>
<td>Retained chromos</td>
<td>Multi-</td>
<td>% Aberrant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung:</td>
<td>200</td>
<td>116</td>
<td>1</td>
<td>82</td>
<td>1</td>
<td>42.0%</td>
<td>41.0%</td>
</tr>
<tr>
<td>(epithelium and connective tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine:</td>
<td>150</td>
<td>84</td>
<td>2</td>
<td>63</td>
<td>1</td>
<td>44.0%</td>
<td>42.0%</td>
</tr>
<tr>
<td>(epithelium and connective tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amnion</td>
<td>1070</td>
<td>854</td>
<td>6</td>
<td>202</td>
<td>8</td>
<td>20.2%</td>
<td>18.8%</td>
</tr>
</tbody>
</table>
In analyzing this table we must state that with respect to embryonic tissue (lung, intestine) there is an almost complete correspondence between the data and the ratio between orthoploid and heteroploid sets (obtained by a count of metaphases) represented in Table 1.

But this is not the case in extra-embryonal tissues. In spite of the presence of 50% of heteroploids, in only 20.2% of the cases can morphological indications of an abnormal distribution of chromosomes be observed. Though here also these deviations might explain the majority of variations in chromosome number, they do not explain all the causes of variation. In any case, our data does not disclose these factors. The possibility of amitosis, for instance, is not excluded, though this cannot be shown in our material. Thus, we come to the conclusion that the chief causes of the variations in the chromosome number are abnormalities in the dynamics of mitosis, which lead to an abnormal distribution of chromosomes among the daughter cells. This phenomenon is based on non-disjunction. In a careful analysis we can often see linked pairs of homologues which move together to one pole. There may be one, two or more of such pairs (see Pl. 15, Figs. 8–12, Text-figs. 19–21). In some figures, it is evident, that chromosome non-disjunction has taken place. Thus Fig. 22 shows two karyological lines of 2 cells of the same amnion. It is clearly seen that in cell “A” one of the largest chromosomes is absent, namely the third on the right. Pl. 15, Fig. 13 shows a tripolar mitosis of a cell of an amnion.

Text-figs. 19–21. 19. The same cell as in Pl. 15, Fig. 13. Non-disjunction and “lagging” of several pairs, chromatinisation of some traction spindle fibers. 20. Another cell of the same amnion. 21. Third cell from the same amnion. Chromatinisation of some traction spindle fibers. “Lagging” of chromosomes.
V. Discussion and Conclusions

Comparing our data with that in the literature we find that our material confirms and partly completes the data pointing to the presence of certain variations in the chromosome number of the embryonic soma (Caffier, Rappeport, Grosser). These data do not concern the law of the individuality of chromosomes or the law of constancy in the chromosome number for the species. With respect to the soma of an adult the question of number constancy remains open at present. The presence of variations in the embryonic soma cannot be interpreted to mean that in general there is no genetic continuity of the chromosomes, or that the individuality of the latter is not preserved. Hypoploids and hyperploids are deviations from the norm which appear in different quantities under specific conditions. Cells which differ in chromosome number from 2n are, in the main, sets of haplosomatic and polysomatic cells for certain chromosomes, a fact which can sometimes be quite clearly proved karyologically.

The most frequent is the orthoploid group of cells. It is not surprising that diploid sets are selected, when the material is comparatively limited and attention is fixed on exclusively “normal”, “well distributed”, “regular” or “symmetric” figures. By this unconscious selection we may explain most of the conclusions of the authors who believe in an absolute (Evans and Swezy) or almost absolute constant chromosome number of the embryonic soma. These authors point out several variations for instance Kemp who out of 25 counts obtained, in two 47, in one 49, and in one 50 chromosomes, accounting for 20% variation, but they did not assign very serious significance to
these facts. In his second paper Kemp admits the possibility of small variations in normal tissues. He writes “Es ist nicht ganz aus-geschlossen, daß auch im normalen Gewebe kleine Schwankungen in der Chromosomenzahl vorkommen” He reaffirmed this during his visit to Moscow in the autumn of 1934. The presence of variations in number is clearly evident in more random and varied material.

From morphological study, it is impossible to say anything concerning the character of the factors leading to mass non-disjunction.

It may be supposed that through intensive tissue metabolism during embryogenesis, different local combinations, exogenous for the cell, may easily arise, and that they cause different abnormalities in the process of cell division. We hope to return to this problem in the future.

It seems probable that the constant presence of heteroploids in embryonic tissue, many of which have lowered resistance and die easily, is a factor which must be taken into consideration in the study of histogenesis. Until recently this factor was disregarded.

In conclusion we may state:

1. Among the cells of the extra-embryonic and embryonic soma in man there are many variations from the diploid number of 48.
2. The variations in our material are from 32 to 73 chromosomes.
3. We could not establish any essential differences between the karyological characteristics of extra-embryonic and embryonic soma.
4. An analysis of chromosome sets within the limits of our work does not show any characteristic peculiarities of differently determined parts in the embryonic soma. The cells of the epithelium, brain, mesenchyme of the skin, lung, and intestine, epithelium of the lung and intestine showed, in the main, a similar karyological picture.
5. Non-disjunction of one or several pairs is the chief factor causing variations in number. Fragmentation, association and elimination may also have some influence.
6. Polypolar mitoses play an insignificant rôle in the development of variations in number.

The present work was undertaken at the initiative of Professor P. I. Jivago, as one of a series of investigations devoted to the problem of the variability of the karyotype during ontogenesis. The authors wish to express their appreciation and thanks for his advice and criticism throughout the work.
References


33) Wilson, E. The cell in development and heredity. 1928.


**Explanation of Plate 15**

Figs. 1–13. Photomicrographs.

Fig. 1. Mesenchyme cell from the villus of the chorion. Prophase. PAINTER-MICHAELIS. 38 chromosomes.

Fig. 2. Brain cell. Prophase. PAINTER-MICHAELIS. a, b, successive focuses of the same section. 73 chromosomes.

Fig. 3. Brain cell. Prophase. PAINTER-MICHAELIS. a, b, c, one section (3 successive focuses) d, e, another section (2 focuses). 51 chromosomes.

Fig. 4. Histiocyte of the skin. Metaphase. PAINTER-MICHAELIS. 32 chromosomes, (29 in one section, 3 in the second).

Fig. 5. Histiocyte of the skin. Metaphase. PAINTER-MICHAELIS. 48 chromosomes.

Fig. 6. Histiocyte of the skin. Metaphase. PAINTER-MICHAELIS. 54 chromosomes.

Fig. 7. Histiocyte of the skin. Prophase. PAINTER-FEULGEN. 50 chromosomes.

Fig. 8. Amnion. Anaphase. FLEMMING-MICHAELIS. Photo. "Retained" chromosomes.

Fig. 9. The same. Another cell.

Fig. 10. The same as above. FLEMMING-FEULGEN. Water-blue. Non-disjunction and lagging of several pairs of chromosomes.

Fig. 11. Amnion. Anaphase. FLEMMING-MICHAELIS. Elimination of chromosomes.

Fig. 12. Amnion. Anaphase. FLEMMING-FEULGEN. Water-blue. "Lagging" separate chromosome.

Fig. 13. Amnion. Metaphase. FLEMMING-MICHAELIS. Tripolar mitosis.
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