THE DISTRIBUTION OF NEWLY SYNTHESIZED DNA IN
MITOTIC DIVISION*

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PLATES 148 AND 149

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The conservative "character of cell reproduction is predicated on the replica-
tion of the genetic material and the precise distribution of the products to the
daughter cells. The grouping of many genetic units in few chromosomes creates
problems in our visualization of the reproductive mechanism as it simplifies
the mechanism itself. The evidence of genetics informs us that at least some
of the smaller units in the chromosome are duplicated as accurately in their
arrangement as in their qualitative activity. The problems of chromosomal
reproduction and of the replication of its molecular units are not necessarily
mutually inclusive; the extent to which the solution of one includes the solution
of the other is an experimental question. It seems desirable to make this point
because the experiments we are reporting were devised to answer a question
concerning chromosome duplication, yet are pertinent to the duplication of a
molecular chromosomal constituent, the deoxyribonucleic acid molecule.

This study was designed to answer this question: When a chromosome repro-
duces, is the material of the parent chromosome equally distributed between
the daughters or does the parent chromosome persist and determine the forma-
tion of a substantially new copy of itself? In terms of the distribution of parental
and newly synthesized material the question can be formulated experimentally
in several ways. A labelling technique can be used. One might label the sub-
stance of the parent chromosome and observe how it is distributed between the
two products of its reproduction. Conversely, the label might be introduced
in the course of the duplication process. Since it is established by other means
(1) that those substances that we recognize as being uniquely constituents of
chromosomes increase exactly twofold in the course of the reproductive process,
the alternative expectations will be the same with either experimental procedure.

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plies of Crepis capillaris seeds, and to Dr. M. Friedkin for generously supplying us with
samples of C14-thymidine.

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If the parent chromosome distributes its substance equally between the daughter chromosomes, these division products will also have to acquire equal amounts of newly synthesized material. If one product is a substantially new copy of the other, one will be labelled and the other unlabelled. A third possibility is that the parental chromosome contributes some, but not half, of its substance toward the formation of a daughter. This would imply that only part of the chromosomal substance is actively involved in the reproductive process.

The answer to this simple question—whether the reproduction of a chromosome involves splitting it—obviously will not tell us how chromosomes duplicate, but the answer is required for the formulation of a mechanism. Since it is one of the few questions about chromosomal reproduction that can be put in experimental form, it is not surprising that experiments similar in principle to those we shall describe have been discussed or attempted previously (2). The execution of the experiment has depended on meeting certain requirements with respect to biological material, method of labelling, and methods of detection and measurement of the label, all of which will be discussed in detail below.

Experimental Design.—Ideally one would wish to label the entire substance of a chromosome (all of its carbon, for instance) and compare the distribution of the label in individual sister chromosomes. In practice, these conditions could not be met, and the experiment was designed as follows:

1. Cells of a plant species with a small number of chromosomes were used. The structure of the plant cell permits the unequivocal identification of sister chromosome groups at anaphase or telophase, before the cell wall has been laid down between sister cells. If the number of chromosomes is sufficiently small, measurement of the distribution of labelled chromosome material between sister groups of chromosomes should meet the requirements of the experiment. If the newly synthesized chromosome material is equally distributed between daughter chromosomes, it must also be distributed equally between daughter nuclei. If at metaphase there is a set of chromosomes containing old material and a set containing new material, and if the separation of sister chromosomes at anaphase is random, then the smaller the number of chromosomes the greater will be the chance of detecting unequal distribution of label between sister chromosome groups. An organism with a suitable chromosome complement and amenability to rapid laboratory culture was found in Crepis capillaris (2n = 6). The meristems of Crepis seedling root tips are characterized by a good mitotic index and take up readily various radioactive precursors that have been introduced into the medium. The chromosome sets of Crepis are large enough to be detected easily by the autoradiographic method and are sufficiently well separated at anaphase and telophase so that the sister chromosome groups may be resolved unequivocally in autoradiographs.

2. DNA was selected as the chromosomal constituent to be labelled. It is a
constant component of chromosomes and is not ordinarily present in other parts of the cell. While there is reason to think that the proteins of chromosomes (especially the histones) may be involved to an important extent in chromosomal structure and function, there is no obvious means of labelling these proteins without labelling other proteins of the same cell. It must be recognized, however, that we are thus limiting ourselves to observing the results of the reproduction of the DNA component and not that of the chromosome substance as a whole.

3. The chromosomal DNA was labelled during the period of synthesis in the interphase preceding the division in which the products were observed. Howard and Pelc (3) have demonstrated by P32 incorporation studies that the DNA synthesis takes place during the interphase and not during the division itself. The same conclusion has been drawn from photometric studies. The time taken by the division process itself is a small fraction of the time required for the complete cycle from one division to the next. If one exposes a population of cells to the isotope-labelled precursor for a period less than that required for the complete cycle and fixes them immediately, then the radioactivity observed in the chromosomes of those cells that are found to be in division is a measure of synthesis of chromosome material during the preceding interphase. Thus, by comparing the radioactivity of sister chromosome groups at anaphase or telophase, we may observe the distribution of the DNA newly synthesized during the reproduction of the parent chromosomes.

(4) The distribution of labelled DNA in sister chromosome groups was observed by the technique of high resolution autoradiography and quantitated by a method described below.

In brief, the experimental design involves the measurement of the distribution between sister sets of chromosomes of the new DNA synthesized in the course of the reproduction of the parent set. The converse experiment, the observation of the distribution of labelled parental chromosome substance, would be instructive but has not yet proved to be practicable.

Methods

DNA Labelling.—Preliminary experiments on the isotopic labelling of DNA in Crepis showed that although standard labels, such as P32O4 and C14 adenine were readily incorporated, the concomitant problem of complete removal of labelled non-DNA components of cell and nucleus presented rather severe difficulties in this material. In order to avoid the need for selective extraction procedures, C14-2-thymine-deoxyriboside (thymidine) was chosen as a label incorporated only into DNA (4).

Duration of Labelling Period.—The time during which a given cell population is exposed to the presence of the labelled material must be long enough to permit a cell which is either synthesizing or about to synthesize DNA when the isotope is introduced to proceed to the analyzable stage, late anaphase or telophase (see Text-fig. 1). Moreover, the time must not be so long as to allow cells to go through two cycles of synthesis and thus obscure possible differences. In the absence of accurate knowledge on the length of the mitotic cycle in Crepis
root meristem cells, the time chosen was 12 hours. Qualitative analysis of the resulting preparations showed that after this period all telophases were labelled; in other words, the value for $x$ in Text-fig. 1 is equal to or less than 12 hours.

Culture of Roots.—Seeds of Crepis capillaris were germinated on water-saturated filter paper. When the roots had attained a length of 5 to 10 mm., the seedlings were transferred to a Stender dish containing a thin layer of cotton, saturated with the isotope solution. The roots were firmly embedded in this cotton to assure good physical contact with the solution which consisted of glass-distilled water in which enough C$^{14}$-thymidine had been dissolved to give a measured specific activity of 0.5 to 0.8 $\mu$c. per ml.

Processing for Autoradiography.—Labelled roots were fixed, after 12 hours of incubation at room temperature and constant light, in acetic alcohol (1:3), macerated in $\pi$ HCl at 60°C. for 3 to 4 minutes (there is no appreciable loss of DNA pyrimidines under these conditions (5)), and squashed on albuminized slides in a drop of 45 per cent acetic acid. After removal of the coverslips (dry ice freezing technique (6)) the slides were passed through alcohol, washed extensively in distilled water, and covered with autoradiographic stripping film (Kodak Ltd., London). Photographic development of the preparations was carried out after 6 and 12 day exposure periods. (For details of autoradiographic procedure see Doniach and Pelc (7).)

RESULTS

Analysis.—The finished preparations were examined first by phase contrast microscopy. When visually clear telophases and anaphases were found they were photographed (contrast process pan emulsion, Eastman Kodak) and the mechanical stage location of the field recorded. These steps were carried out with the autoradiographic emulsion above the tissue hydrated and covered with a coverslip. For the subsequent observation of the autoradiographs the emulsion was permitted to dry (reducing the thickness of the emulsion and thus the depth over which the silver grains are distributed), covered with immersion oil and a clean coverslip, and studied in bright field with an objective of suit-
Text-Fig. 2. Diagrammatic illustration of the preparation of a bright-field negative for scanning. A, phase contrast photograph of a telophase. B, bright-field photograph of the autoradiograph of the telophase. Since the two photographs are taken at the same magnification, they can be superimposed and lines a, b, c, and d, cut into the bright-field negative to mark the limits of chromosomal detail, as indicated. The marked negative B is then placed in the mask C so that the significant grain patterns fall into the clear path of width W. This width represents the maximum value for the ana- and telophase figures analyzed. The mask C is an integral part of the scanning apparatus shown in Text-fig. 3. (It corresponds to the item labeled b there.) It should be noted that the actual bright-field negative used in the analysis differs from that illustrated by B in color relationship: its grains are light-transmitting areas on a dark background.
Text-Fig. 3. Diagram of scanning apparatus. a, light source. b, negative holder and mask. c, phototube slit, end view. d, gas-filled phototube. e, impedance matching device. f, amplifier and recorder (Hazotrol Corporation, Palo Alto; Servo unit HC 100 and chart drive HC 10, operated through flexible shaft (g) by motor (i), thus synchronized with negative holder (b)). g, flexible drive shaft. h, device for vertical movement of negative holder. i, variable speed motor. j and k, variacs.

Text-Fig. 4. Sample scanning record of a telophase autoradiograph. The jagged peaks are the positions of the cuts in the negative. The shaded areas are the measure of light transmission due to the two halves of the telophase. Their weights in milligrams are noted. The resulting ratio: $\frac{73.7}{36.7} = 2.0:1$. The basis of a conservative error in the ratios is evident here: the base line is drawn at the lowest background level for the space between the two telophase halves; it is very probable that this leads to the inclusion of some background in the two measurements and therefore a depression of the real ratio. The record is that corresponding to entry D-1 on Table I.
TABLE I

Relative Amounts* of Newly Synthesized DNA at the Two Poles of Anaphase and Telophasic Cells

<table>
<thead>
<tr>
<th>Measurement No.</th>
<th>Relative amounts</th>
<th>Ratio</th>
<th>Exposure time</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-1a</td>
<td>59.4:50.6</td>
<td>1.17:1</td>
<td>6</td>
</tr>
<tr>
<td>B-1b</td>
<td>69.2:63.5</td>
<td>1.09:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>B-2</td>
<td>82.1:67.2</td>
<td>1.22:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>B-4</td>
<td>70.8:69.2</td>
<td>1.02:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>C-3-1</td>
<td>40.0:38.9</td>
<td>1.06:1</td>
<td>6</td>
</tr>
<tr>
<td>C-3-2</td>
<td>54.7:24.7</td>
<td>2.22:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>C-5</td>
<td>90.2:42.2</td>
<td>2.12:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>C-8</td>
<td>52.9:42.6</td>
<td>1.24:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>C-9</td>
<td>91.8:59.2</td>
<td>1.53:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>A-1</td>
<td>168.0:67.0</td>
<td>2.36:1</td>
<td>12</td>
</tr>
<tr>
<td>A-4</td>
<td>237.8:96.2</td>
<td>2.47:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>Adjoining prophase</td>
<td>343.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-5</td>
<td>93.2:86.7</td>
<td>1.07:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>A-8</td>
<td>140.6:106.9</td>
<td>1.32:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>D-1</td>
<td>73.7:36.7</td>
<td>2.00:1</td>
<td>12</td>
</tr>
<tr>
<td>D-2a</td>
<td>88.9:72.1</td>
<td>1.23:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>D-2b</td>
<td>156.1:92.7</td>
<td>1.68:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>D-4</td>
<td>134.8:85.9</td>
<td>1.57:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>D-5</td>
<td>77.4:55.9</td>
<td>1.38:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>D-7</td>
<td>75.3:35.0</td>
<td>2.15:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-1a</td>
<td>175.4:136.2</td>
<td>1.29:1</td>
<td>12</td>
</tr>
<tr>
<td>E-1b</td>
<td>238.6:206.4</td>
<td>1.15:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-1c</td>
<td>170.4:146.0</td>
<td>1.17:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-1e</td>
<td>137.5:142.1</td>
<td>1.03:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-1f</td>
<td>141.2:107.8</td>
<td>1.31:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-2</td>
<td>158.6:79.0</td>
<td>2.00:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-3</td>
<td>134.2:102.1</td>
<td>1.32:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-4</td>
<td>191.0:91.0</td>
<td>2.10:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-5</td>
<td>217.7:210.0</td>
<td>1.04:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-6</td>
<td>143.6:82.4</td>
<td>1.74:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>E-7</td>
<td>82.4:69.1</td>
<td>1.19:1</td>
<td>&quot;</td>
</tr>
<tr>
<td>Adjoining prophase</td>
<td>152.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as area under transmission curves obtained by scanning (see text for methodology). Area is given here in milligrams of paper. Comparisons of weights are valid only within one measurement. The ratios are comparable throughout.

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anaphases and telophases were relocated with the aid of the mechanical stage and by the over-all correspondence between grain clusters and nuclei (see Figs. 1 to 4). The autoradiographs of previously identified cells were then photographed with Koehler illumination and a high contrast emulsion (kodalith ortho, type B, Eastman Kodak; see Mazia et al. (8), for further discussion). The phase contrast photograph of the cells and the bright-field photograph of the autoradiograph were taken at the same final magnification. The two negatives of a given field could thus be superimposed and the outline of a cell as well as the location of chromosome groups indicated on the bright-field negative, re-

Text-Fig. 5. Frequency distribution of the ratios obtained from the 30 anaphases and telophases measured. (Ratio values rounded off to nearest tenth unit.)

<table>
<thead>
<tr>
<th>Exposure time</th>
<th>No. of cases</th>
<th>Average ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>1.41:1</td>
</tr>
<tr>
<td>12</td>
<td>21</td>
<td>1.50:1</td>
</tr>
</tbody>
</table>

sulting in a clear definition of the areas containing pertinent grain images (see Text-fig. 2 for a diagrammatic visualization of this process). The marked bright-field negative was then placed in the path of a light beam focused on a gas-filled photocell, in a rack capable of uniform movement. As the phototube was masked except for a narrow horizontal slit, the amount of light acting on it at any one moment represented a summation of light spots (grain images) in the negative across the width of the scanning path, with a small longitudinal component defined by the width of the phototube slit. The phototube signal was amplified and recorded by an automatic instrument. (See Text-fig. 3 for details of the scanning and recording arrangement.) The final record of a scanned negative Text-fig. 4) shows spikes where the negative had been cut to indicate
boundaries and smooth curves between these spikes, representing the light transmission of pertinent grain images. The quantitative analysis was completed by evaluating the area under the curves, in terms of their weight, and expressing the result per cell as the ratio between the two weights.

Summary of Results.—The ratios obtained from the analysis of 30 cells in anaphase or telophase are listed in Table I, together with exposure time of the autoradiographic emulsion for the respective slide. In Table II the average ratio for each of the two exposure periods used is given. The frequency distribution of the 30 ratios is shown graphically in Text-fig. 5. The existence of a large number of ratios higher than 1.0:1.0 leads to the possible conclusion that new DNA need not be equally distributed between the products of mitotic division.

DISCUSSION

Possible Sources of Experimental Error.—The principal question which arises out of the apparent inequality of newly synthesized DNA in the two halves of a postmetaphase cell concerns, of course, the significance of the measured inequalities. In other words, could the newly synthesized DNA be distributed equally and still lead to the data reported here? We are concluding that it could not. This conclusion arises from a study of the errors which could be involved in the data obtained.

These errors fall into three major categories: (a) lack of constant proportionality between grain numbers in the autoradiographic emulsion and new DNA synthesized; (b) a geometrical error; (c) errors involved in the quantitation procedure.

The lack of constant proportionality between new DNA and grains could be the result of (a) unequal labelling of equal amounts of new DNA, (b) unequal decay of equal pools of C14 atoms, or (c) non-uniform emulsion response. The possibility of unequal labelling appears to us very remote, particularly in view of the enormous number of C14 atoms involved. A reasonably constant proportionality may also be assumed for the number of grains per unit of label, since a doubling of the number of grains, through prolonged exposure, does not lead to significantly different ratios (see Table II). The actual number of grains was roughly estimated at about 110 for the average metaphase cell after 6 days of exposure. This would lead to an estimated average of about 220 grains for a 12 day exposure metaphase autoradiograph. It is very unlikely that the random distribution of the C14 atoms corresponding to these grain numbers would result in the observed distribution of ratios. There is no basis from past experience with the autoradiographic emulsion employed for the assumption of serious non-uniformity in the beta sensitivity of adjacent regions. Within the present experiment we encountered two cases where the autoradiographs over postmetaphase cells could be directly compared with those over adjacent
premetaphase cells. In both cases the summed measurements of the division products showed close agreement with the predivision nuclei (see Figs. 3 and 4). This measured equality, where equality is expected, furnishes some concrete evidence against serious non-uniformity in labelling and film response. A more extensive test for equality could be based on the comparison of the total label of all telophase cells. Such a test cannot be applied to the present data since the absolute values of the measurements are a function of not only the grain number but also the light intensity used in the scanning process. This light intensity was varied between measurements to maintain a uniform sensitivity level with negatives of varying over-all density. (The high contrast of the kodalith emulsion makes the preparation of a large number of negatives with equal density exceedingly difficult.) Valid comparisons of the existing data are therefore restricted to measurements made on a single negative, where light intensity was kept constant. Aside from the measurements used to calculate the ratios, only the two aforementioned cases of premetaphase cells fall within this group.

A geometrical error could arise if the two halves of an anaphase or telophase were separated from the emulsion by appreciably different distances. If this were the case, the near half would produce a more concentrated grain pattern than that further removed, the latter a relatively diffuse pattern, portions of which could fall outside the scanning path and thus be lost. While the actual distances which could be involved are too small to lead to the loss of beta particles by absorption, they could be large enough to introduce an error due to the scattering of these particles. Careful observation of the autoradiographs (cf. Figs. 1 to 3) suggests that this factor is probably not operative to a significant degree in our preparations: the poor autoradiographic resolution which would be a consequence of greater separation is not obviously present. We have seen no cases where an autoradiographic pattern could not be clearly circumscribed within the limits of the scanning path.

The errors involved in quantitation are of three types. The first concerns the accuracy with which the autoradiograph of a single nucleus can be measured. We have found this error to be of the order of 3 per cent (8) (measured in other material). The second type of analytical error arises from the existence of a background grain density in the emulsion which is at least partly independent of the experimentally significant grain density produced by the C14 beta particles. The measurement of each half of an anaphase or telophase will include a certain amount of this component. On the assumption that this background is relatively uniform, both halves will contain about the same amount. If we denote the significant grain density for the two halves of a postmetaphase cell by $A^1$ and $A^2$ and the background density by $B$, then the measured values for the two halves will be $A^1 + B$ and $A^2 + B$. Because of its general simplicity we have chosen the ratio as the best method for expressing the results of the
measurement. The distribution of labelled DNA in a postmetaphase cell is thus expressed as $A_1^1 + B : A_2^2 + B$. If $A_1^1$ and $A_2^2$ are nearly equal or equal, the value of $B$ will have little or no effect on the value of the quotient. However, as $A_1^1$ and $A_2^2$ become more unequal, the influence of $B$ on the quotient becomes progressively more pronounced. This influence will shift the ratio value in the direction of unity, towards that value expected from an equal distribution of labelled material. In the analysis of the curves obtained with our measuring system we have assumed the background grain density to be relatively low. This is illustrated by the position of the base of the shaded areas in Text-fig. 4. It is very probable that the true background density is higher and that we have included appreciable amounts of background component in the measured values. Consequently the ratios derived from these measurements should be regarded as conservative. Although this error will obviously affect the over-all range and distribution of ratios, it does not invalidate the hypothesis that the measurements show real inequality.

A third source of quantitation error may be present in autoradiographs which are sufficiently dense so that grains overlie each other and do not contribute fully to the measurement made. This would lead to a depressed ratio where inequality exists since the more heavily labelled half of an anaphase or telophase would be more subject to this underestimate than its less heavily labelled sister half. This density effect can lead to a mistaken conclusion of inequality only when two equally labelled halves of a postmetaphase cell give rise to autoradiographs of unequal areal distribution. The measurement could then give a low value for the more concentrated autoradiograph and a higher value for the less concentrated. Such cases would have had to occur with an appreciable frequency to lead to the ratios reported. We have found no cells whose measurement indicated pronounced inequality and where the lower value corresponded to a more concentrated autoradiograph. It is therefore highly unlikely that the measured inequality is the result of this type of error. We cannot, however, rule out the possibility that some of the more heavily labelled anaphase and telophase halves have been underestimated because of this effect. If this error is present it provides another reason for regarding the ratios obtained as conservative.

Obviously there is a need for further data to permit a statistical evaluation of the effect of factors which are discussed here only in qualitative terms. Experiments designed to provide the basis for such tests are in preparation.

The Fate of Newly Synthesized DNA.—The conclusion that there occur unequal amounts of new DNA in the products of one nuclear division seems to us the most probable one. In view of the fact that the total amounts of DNA in the division products differ by less than 10 per cent (9) (measured in other material), this conclusion can be taken one step further: the proportion between new and old DNA is not the same for the two nuclear division products of all
cells. It follows that, contrary to the suggestion of Daoust et al. (10), DNA synthesis, at least with respect to thymidine, does not involve the reconstitution of all the DNA, but that there are two "types," new and old, labelled and unlabelled. Insofar as DNA represents the chromosome we have, then, an answer to the initial question: the parental material is not equally distributed between daughter chromosomes.

The Number of Strands per Chromosome.—We may now proceed to an attempt to define the number of strands of which a Crepis anaphase chromosome consists. By strand we mean that DNA-containing biosynthetic unit which separates discretely after metaphase. It is that element of the anaphase chromosome which can be regarded as old or new with respect to DNA. The strand is thus defined functionally and no assumptions are made concerning its microscopic appearance or its quantitative characteristics at the molecular level. It may correspond to any number of DNA chains that are synthesized and segregate together. The expected distribution pattern of new DNA between the two poles of anaphases and telophases can be calculated for the Crepis system of 6 chromosomes. With 1 strand per chromosome, we would expect a total of 6 labelled and 6 unlabelled elements per telephase cell, and the random distribution of labelled units would lead to the ratio classes 6:0, 5:1, 4:2, and 3:3, with frequencies of, respectively, 0.03, 0.19, 0.47, and 0.31. If we assume more units per chromosome the number of classes will obviously increase until, with an infinite number of units, the distribution will be that described by a smooth normal curve. In view of the uncertainty of individual ratio values the most critical method of analysis would depend on the comparison of observed all:nothing ratios with the expected for the several models. A sample of 30

### TABLE III

*Expected Distribution Frequencies of Ratios of New Chromosomal Units in Postmetaphase Cells, with Varying Numbers of Such Units per Anaphase Chromosome*

(Calculated on the basis of a binomial distribution \((a + b)^n\), in which \(a = b = \frac{1}{2}\) and \(n\) varies from 6 to 24, corresponding to from 1 to 4 units per anaphase chromosome.)

<table>
<thead>
<tr>
<th>No. of units</th>
<th>Expected frequency of ratio in anaphase or telophase</th>
<th>Expected out of 30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:1 to 1.9:1</td>
<td>2:1 or higher</td>
</tr>
<tr>
<td>1</td>
<td>0.31</td>
<td>0.69</td>
</tr>
<tr>
<td>2</td>
<td>0.61</td>
<td>0.39</td>
</tr>
<tr>
<td>3</td>
<td>0.76</td>
<td>0.24</td>
</tr>
<tr>
<td>4</td>
<td>0.846</td>
<td>0.164</td>
</tr>
<tr>
<td>Observed</td>
<td></td>
<td>22</td>
</tr>
</tbody>
</table>

Thus, the observed distribution of ratios is compared with the expected distribution for different numbers of units per chromosome.
ratios is probably insufficient for this purpose. This sample may suffice, how-
however, to give an indication if we consider only ratios of 2:1 or greater (high) and
less than 2:1 (low) and their relative frequencies. The relative frequencies of
these ratio groups for strand numbers from one to four have been tabulated in
Table III. It is evident from this table that the proportion of high ratios
decreases with increasing strand numbers. The observed distribution of 22 low
to 8 high ratios, falls below that expected for 4 anaphase chromosomal strands,
and would appear to fit the 3 strand model fairly well. However, the afore-
mentioned measuring errors, which would tend to depress high ratios, lead to
the possibility that the true distribution is less than 22 low to more than 8 high
ratios in a sample of 30. The possibility of 2 strands per anaphase chromosome
in Crepis capillaris is therefore not contradicted by the data. Even a single
strand chromosome is conceivable as consistent with our findings in view of the
fact that we have not taken into account the possibility of segmental inter-
ruption of strands during or after synthesis. Such a phenomenon could lead to
the intermediate values which, a priori, would not be expected if only a single
synthetic unit per chromosome strand were involved.

While this clearly speculative interpretation of the data leaves considerable
uncertainty as to the exact number of strands, this number is probably less
than 4; the analytical errors would not exaggerate the frequency of high ratios.
Only the assumption of non-randomness in the separation of newly synthesized
strands, i.e. a preference among newly synthesized units to move together,
could reconcile a larger number of units with the data obtained. One limitation
of our method is its inability to detect this type of non-randomness. It was for
this reason that the term strand was defined as an independently behaving unit
of synthesis. In addition, a more refined analysis of strand number would have
to take into account the difference in size and probable difference in DNA
content of the chromosomes of Crepis capillaris.

This analysis of strand number is based on a rather literal interpretation of
the data. The conclusions drawn are obviously subject to modification in the
event that further work shows the experimental errors to be greater than
anticipated.

Chromosome Duplication and DNA Duplication.—The conclusion that
parental DNA is not equally distributed between the products of chromosomal
duplication is obviously insufficient to postulate a mechanism of duplication
of the whole chromosome. DNA is only one of the molecular constituents of the
chromosome. On the other hand, this conclusion can be used in a critical
examination of one of the hypotheses advanced on the duplication of the DNA
molecule. The most plausible theory of DNA duplication has been that sug-
gested by Watson and Crick (11). According to this scheme, a DNA molecule
duplicates by the separation of the two complementary helices of which it is
composed; each helical strand then determines the synthesis of a new helical strand which is complementary to it. If the kind of experiment that we have described for the chromosome were applied to the DNA molecule, the Watson-Crick scheme would predict that each daughter molecule would consist half of old material and half of new material. If a population of DNA molecules reproduced thus, the label would be distributed uniformly among the product molecules. Therefore, if every DNA molecule of a chromosome set duplicated by the Watson-Crick scheme, the predicted results would be contrary to those we have actually observed. The Watson-Crick scheme appears incompatible with the assumption that every molecule of the chromosome reproduces. (We would like to emphasize that our data have no bearing on the structure of the DNA molecule proposed by Watson and Crick (12) but only on the mechanism of its duplication.)

It is necessary, therefore, either to find an alternate mechanism for DNA duplication that would eliminate the permanent separation of the 2 strands of the double helix or to assume redundancy of chromosomal DNA such that only part of the DNA is involved in the reproductive process. We shall not discuss here the details of various alternatives to the Watson-Crick mechanism that have been proposed (13). One class of such schemes in which the DNA is duplicated indirectly through an intermediate agent such as protein or RNA is attractive because it has some experimental support (14). It is not immediately obvious how the question of redundancy of DNA in the chromosomes may be tested.

SUMMARY

The chromosomes of Crepis capillaris were labelled with thymidine-2-C\textsuperscript{14} in their DNA fraction. Quantitative analysis of the distribution of newly synthesized DNA in postmetaphase stages of the division following the period of label incorporation led to the conclusion that the new DNA is not necessarily equally distributed by the mitotic process and that, therefore, chromosome duplication does not involve the equal partition of parental DNA. The implications of these findings with respect to DNA duplication are discussed. An attempt is made to translate the pattern of new DNA distribution into a probable number of units of synthesis per chromosome.

BIBLIOGRAPHY

EXPLANATION OF PLATES

PLATE 148

Figs. 1 A, 2 A, 3 A. Phase contrast photomicrographs of fields containing distinct anaphases and telophases. Figs. 1 B, 2 B, 3 B, are the corresponding bright-field photomicrographs of the autoradiographic emulsion. The correspondence between nuclei and grain clusters should be noted.

The dark horizontal lines in Figs. 1 B, 2 B, and 3 B are cuts made in the photograph of the autoradiographs prior to scanning as explained elsewhere.

The results of the quantitative analysis of these preparations are as follows:
- Telophase in Fig. 1: 91 and 191, a ratio of 2.1:1.
- Telophase in Fig. 2: 144 and 82, a ratio of 1.8:1.
- Anaphase in Fig. 3: 82 and 69, a ratio of 1.2:1.
- Prophase in Fig. 3: 153.9 (note good agreement with summed anaphase values).

Figs. 1, 2, and 3 correspond, respectively, to entries E-4, E-6, and E-7 in Table I. Magnification 576.
(Plaut and Mazia: DNA distribution in mitotic division)
PLATE 149

Figs. 4 A and B. Phase contrast and bright-field photographs of a telophase and adjoining prophase. The quantitative evaluation of the three grain clusters resulted in 238 and 96 for the telophase (a ratio of about 2.5:1) and 343+ for the prophase. The good agreement between the summed telophase values with that of the prophase should be noted. These figures correspond to entry A-4 in Table I. Magnification 1600.
(Plaut and Mazia: DNA distribution in mitotic division)