THE ORGANIZATION AND DUPLICATION OF CHROMOSOMES AS REVEALED BY AUTORADIOGRAPHIC STUDIES USING TRITIUM-LABELED THYMIDINE

By J. Herbert Taylor,* Philip S. Woods, and Walter L. Hughes

DEPARTMENT OF BOTANY, COLUMBIA UNIVERSITY; BIOLOGY DEPARTMENT AND MEDICAL DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY

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Information on the macromolecular organization of chromosomes and their mode of duplication has been difficult to obtain in spite of numerous attempts. One point of attack, long recognized but until recently unattainable, was the selective labeling of some component of the chromosome, the distribution of which could be seen in succeeding cell divisions. Reichard and Estborn[1] demonstrated that N^{15}-labeled thymidine was a precursor of deoxyribonucleic acid (DNA) and that it was not diverted to the synthesis of ribonucleic acid. Recently Friedkin *et al.*[2] and Downing and Schweiger[3] have used C^{14}-labeled thymidine to study DNA synthesis. In chick embryos and *Lactobacillus* there was no appreciable diversion of the tracer to ribonucleic acid. In view of these findings, thymidine appeared to be the intermediate required for the experiment, but the labels so far employed have not been satisfactory for microscopic visualization by autoradiographic means. In order to determine whether an individual chromosome among several in a cell is radioactive, autoradiographs with resolution to chromosomal dimensions must be obtained. Resolution at this level is difficult if not impossible to obtain with most isotopes, since the range of their beta particles is relatively great. Theoretically tritium should provide the highest resolution obtainable, since the beta particles have a maximum energy of only 18 Kev, corresponding to a range of little more than a micron in photographic emulsions. Consequently, identification of this label in particles as small as individual chromosomes should be possible. With this in mind, tritium-labeled thymidine was prepared and used to label chromosomes and to follow their distribution in later divisions by the use of photographic emulsions.

Materials and Methods.—Tritium-labeled thymidine of high specific activity (3×10^3 mc/mM) was prepared by catalytic exchange of tritium from the carboxyl group of acetic acid to a carbon atom in the pyrimidine ring of thymidine (details of the method to be described elsewhere).

Seedlings of *Vicia faba* (English broad bean) were grown in a mineral nutrient solution containing 2–3 μg/ml of the radioactive thymidine. This plant was selected because it has 12 large chromosomes, one pair of which is morphologically distinct, and because the length of the division cycle and the time of DNA synhesisl in the cycle are known.[4] After growth of the seedlings in the isotope solution for the appropriate time, the roots were thoroughly washed with water and the seedlings were transferred to a nonradioactive mineral solution containing colchicine (500 μg/ml) for further growth. At appropriate intervals roots were fixed in ethanol–acetic acid (3:1), hydrolyzed 5 minutes in 1 *N* HCl, stained by the Feulgen reaction, and squashed on microscope slides. Stripping film was applied, and autoradiographs were prepared as described previously.[5]

Experimental Design and Results.—Roots remained in the isotope solution for 8 hours, which is approximately one-third of the division cycle.[4] Since about 8 hours intervene between DNA synthesis in interphase and the next anaphase, few if any nuclei which had incorporated the labeled thymidine should have passed through a division before the roots were transferred to the colchicine solution. In the presence of colchicine, chromosomes contract to the metaphase condition, and the sister chromatids (daughter chromosomes), which ordinarily lie parallel to each other, spread apart. The sister chromatids remain attached at the centromere region for a period of time, but they finally separate completely before transforming into an interphase nucleus. Because colchicine prevents anaphase movement and the formation of daughter cells, but does not prevent chromosomes from duplicating, the number of duplications following exposure to the isotope can be determined for any individual cell by observing the number of chromosomes. Cells without a duplication after transfer to colchicine will have the usual 12 chromosomes at metaphase (c-metaphase), each with the two halves (sister chromatids) spread apart but attached at the centromere. Cells with one intervening duplication will contain 24 chromosomes, and those with two duplications will contain 48 chromosomes.

Two groups of roots were fixed. The first group remained in the colchicine solution 10 hours. The second group remained in the colchicine for 34 hours. In the first group, cells at metaphase had only 12 chromosomes, which indicated that none of these had duplicated more than once during the experiment. The chromosomes in these cells were all labeled, and, furthermore, the two sister chromatids of each chromosome were equally and uniformly labeled (Fig. 1, *a* and *b*). The amount of radioactivity in the chromosomes varied from cell to cell, as would be expected in a nonsynchronized population of cells, but within a given cell the label in different chromosomes was remarkably uniform.

In the second group, cells contained either 12, 24, or 48 chromosomes. Those with 12 chromosomes usually were not labeled, but when labeling occurred, sister chromatids were uniformly labeled as in the first group. In cells with 24 chromosomes, all chromosomes were labeled; however, only one of the two sister chromatids of each was radioactive (Fig. 2, *a* and *b*). Evidently the pool of labeled precursor in the plant had been quickly depleted after the plant was removed from the isotope solution, and these cells with 24 chromosomes had gone through a second duplication in the absence of labeled thymidine.

In the few cells with 48 chromosomes, analysis of all 48 was not possible. However, in several cases where most of the chromosomes were well separated and flattened, approximately one-half of the chromosomes of a complement contained one labeled and one nonlabeled chromatid, while the remainder showed no label in either chromatid. The appearance of cells with 48 chromosomes in a 34-hour period in colchicine also indicates that there was some variation in the predicted 24-hour division cycle.

In cells with 24 and 48 chromosomes a few chromatids were labeled along only a part of their length, but in these cases the sister chromatids were labeled in complementary portions (Fig. 2, *b*, *arrow*). This is the expected situation following sister chromatid exchange and demonstrates that resolution is sufficient to see crossing over in cytological preparations. A careful search of numerous cells with 12 chromosomes failed to yield a decisive case of half-chromatid exchange, which

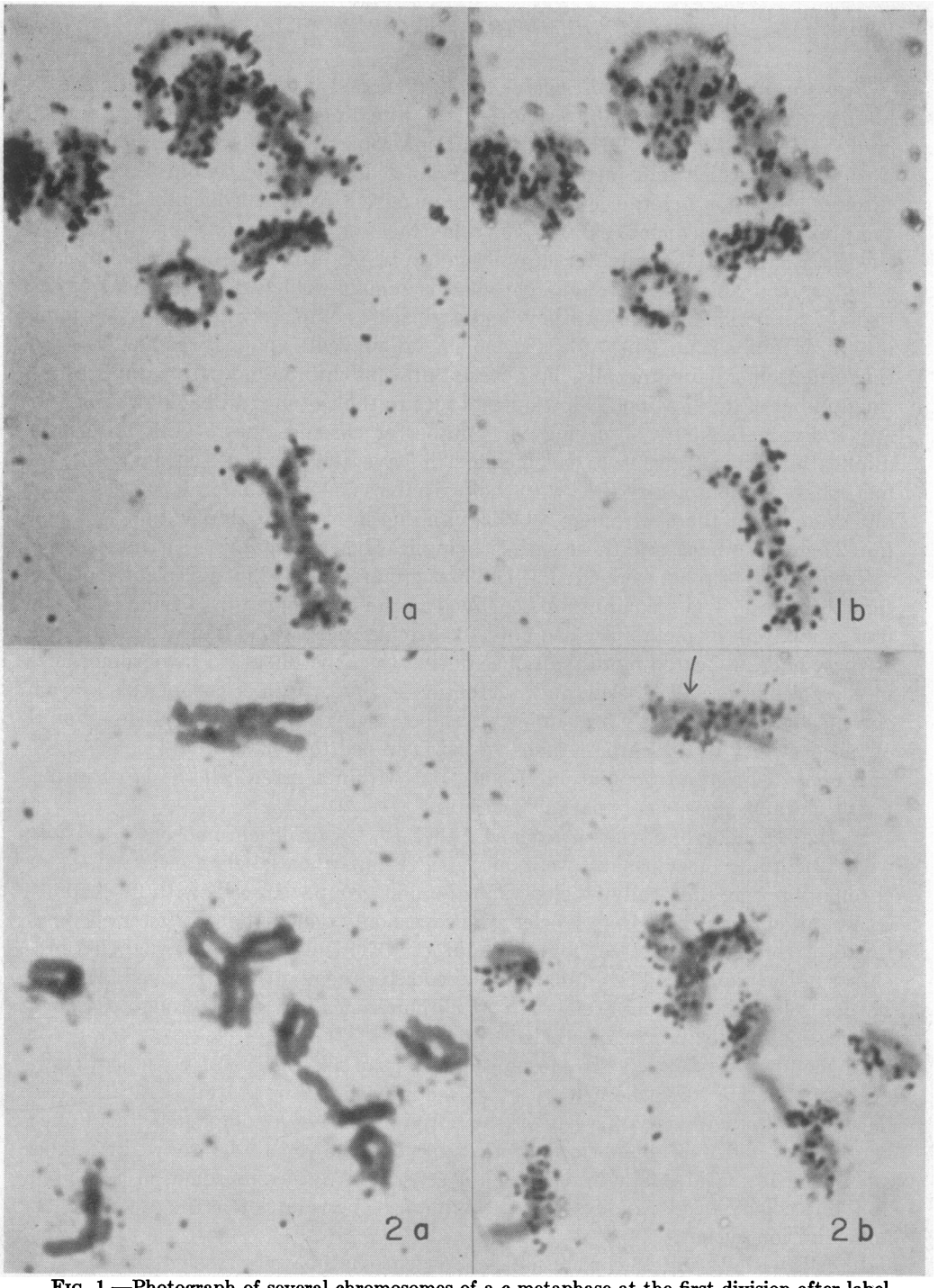

FIG. 1.—Photograph of several chromosomes of a c-metaphase at the first division after labeling occurred; *a*, chromosomes with the chromatids spread apart but still attached at the centromere; *b*, grains in the emulsion above the chromosomes. ×2,200.

FIG. 2.—Photograph of several chromosomes after labeling and one replication in the absence of labeled precursor; *a*, several of the chromosomes from a cell containing 24 chromosomes with chromatids spread but attached at the centromere; *b*, grains in the emulsion above the chromosomes in *a*. ×2,200.

should produce a portion of a chromatid without a label at the first c-metaphase following incorporation of the isotope.

Interpretation and Discussion.—These results indicate (1) that the thymidine built into the DNA of a chromosome is part of a physical entity that remains intact during succeeding replications and nuclear divisions, except for an occasional chromatid exchange; (2) that a chromosome is composed of two such entities probably complementary to each other; and (3) that after replication of each to form a chromosome with four entities, the chromosome divides so that each chromatid (daughter chromosome) regularly receives an "original" and a "new" unit. These conclusions are made clearer by the diagrams in Figure 3. Beginning with two complementary nonlabeled strands in a chromosome, the two strands separate and a complementary labeled strand is produced along each original strand. At the succeeding metaphase each chromatid would appear labeled, although it contains both a labeled and a nonlabeled strand. At a succeeding replication in the absence of labeled precursor, each strand would have a nonlabeled complementary strand produced along its length. At the succeeding metaphase only one chromatid of each chromosome would appear labeled. Following another replication, only one-half of the chromosomes would contain a labeled chromatid, as demonstrated in those cells with 48 chromosomes.

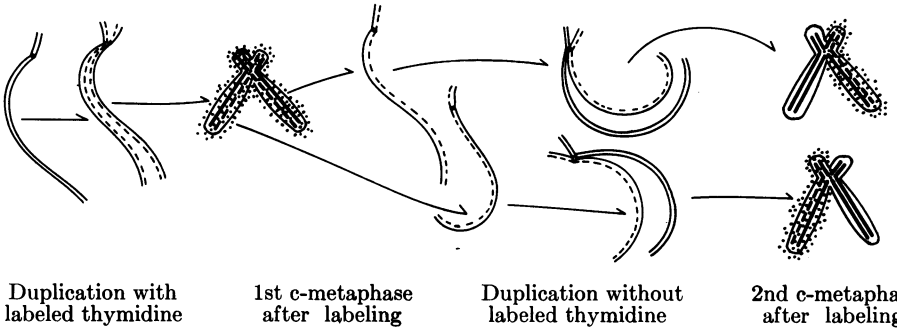

FIG. 3.—Diagrammatic representation of proposed organization and mode of replication which would produce the result seen in the autoradiographs. The two units necessary to explain the results are shown, although these were not resolved by microscopic examination. Solid lines represent nonlabeled units, while those in dashed lines are labeled. The dots represent grains in the autoradiographs.

It is immediately apparent that this pattern of replication is analogous to the replicating scheme proposed for DNA by Watson and Crick.[6] We cannot be sure, of course, that separation of the two polynucleotide chains in the double helix is involved, for the chromosome is several orders of magnitude larger than the proposed double helix of DNA.

We know that these large metaphase chromosomes are coiled into at least one helix at the microscopic level and perhaps are twice coiled, a helix within a helix. That the chromosome could be a single supercoiled double helix of DNA is inconceivable when one considers the amount of DNA in a large chromosome. Chromosomes are much more likely to be composed of multistranded units. To explain their duplication as well as their mechanical properties at the microscopic level, they may be visualized as two complementary multistranded ribbons lying flat

upon each other, as shown in Figures 4 and 5. Ribbons of this type with more flexible materials on their outer edges will have a tendency to coil. If the edges contract faster than the central strands when the chromosome begins to shorten, the ribbons fold, one within the other, so as to form a long, trough-shaped cylinder (Fig. 4), and with further contraction they assume the form of a helix. Continued differential contraction would produce a helix within a helix, but the mechanical properties of the model are outside the scope of this discussion.

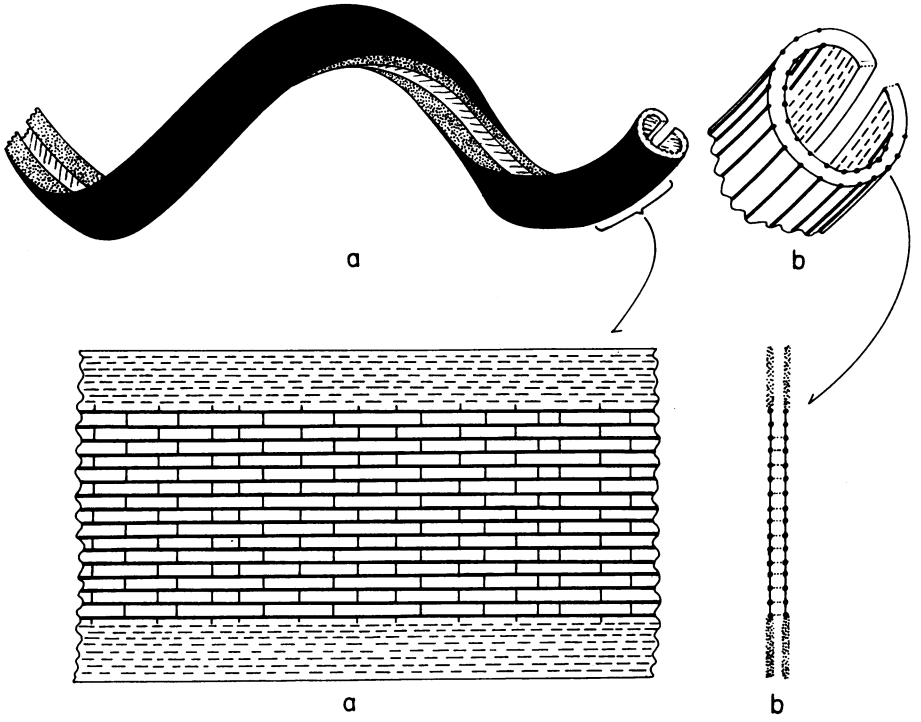

FIG. 4.—Schematic drawing of the proposed ribbon-shaped chromosome with the two multistranded units folded together and coiled; *a*, a single gyre from the coiled chromosome; *b*, detail in cross-section.

FIG. 5.—Diagrammatic sketch of the multistranded units uncoiled and flattened; *a*, short portion in longisection; *b*, cross-section. The number and size of strands shown have no special significance. Although the assumption is made that the strands contain DNA, they do not necessarily correspond to Watson-Crick double helices.

Although the chromosome model is provisional and may require considerable modification and refinement, it has many of the features necessary for duplication and the known stability of genetic materials. The large surface area exposed when the two complementary ribbons are extended would facilitate their rapid duplication. A double-stranded unit with two complementary faces has a high stability,[7] and if the two complementary units are composed of multiple, identical strands cross-bonded, the stability of the larger units should be even greater. Such large units would have a high probability of being transmitted as physical entities. If separation of the complementary faces involves the separation of intertwined double helices of DNA, unwinding presents a problem, but perhaps not an impossible one.[8]

The findings reported here are consistent with those recently reported for the distribution of P^{32}-labeled phage DNA by Levinthal.[9] His data indicated that about 40 per cent of phage DNA is contained in one piece which is divided equally in the formation of two daughter particles, but undergoes no further distribution during the production of about 150 particles that result from the infection of a bacterium. A phage particle would be analogous to a chromosome before duplication, and the first two daughter particles, each of which would be labeled, would be analogous to the two sister chromatids. Since the chromosome is much larger and contains much more DNA than the phage, it is remarkable that they behave in a similar manner in distribution of their DNA during replication.

Our findings are at variance with the report by Mazia and Plaut[10] which was based on the analysis of the anaphase distribution of chromosomes labeled with C^{14}-thymidine. Their data, obtained by the estimation of number of grains over pairs of anaphase or telophase nuclei, indicated a segregation of labeled and nonlabeled units at the first division following the incorporation of the isotope. It is entirely possible that in their experiment more than one division occurred between the time of incorporation and the time the telophase nuclei were analyzed. If this had been the case in the present experiment, unequal distribution of activity in sister chromatids would have been observed in diploid cells.

Summary.—Tritium-labeled thymidine was prepared and used for labeling chromosomes during their duplication. Analysis of autoradiographs showed that both daughter chromosomes resulting from duplication in the presence of labeled thymidine appeared equally and uniformly labeled. After an ensuing duplication in the absence of the labeled DNA precursor, the label appeared in only one of each two chromatids (daughter chromosomes). These findings indicate that DNA is synthesized as a unit which extends throughout the length of the chromosome. The units remain intact through succeeding replications and nuclear divisions, except for occasional chromatid exchanges. Each chromosome is composed of two such units, probably complementary to each other. After each replication the four resulting units separate, so that each daughter chromosome always contains an "original" and a "new" unit. To explain the results, a model with two complementary units and a scheme of replication analogous to the Watson-Crick model of DNA is proposed.

* This work was initiated and the original experiments carried out while the senior author was a research collaborator in the Biology Department, Brookhaven National Laboratory. It has been continued at Columbia University under Contract AT (30-1)-1304 with the Atomic Energy Commission.

[1] P. Reichard and B. Estborn, *J. Biol. Chem.*, **188**, 839, 1951.

[2] M. Freidkin, D. Tilson, and D. Roberts, *J. Biol. Chem.*, **220**, 627, 1956.

[3] M. Downing and B. S. Schweigert, *J. Biol. Chem.*, **220**, 521, 1956.

[4] A. Howard and S. R. Pelc, *Exptl. Cell Research*, **2**, 178, 1951.

[5] J. H. Taylor and R. D. McMaster, *Chromosoma*, **6**, 489, 1954.

[6] J. D. Watson and F. H. C. Crick, *Nature*, **171**, 964, 1953; *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 123, 1953.

[7] H. Kacser, *Science*, **124**, 151, 1956.

[8] N. Ardley, *Nature*, **176**, 465, 1955; M. Delbrück, these PROCEEDINGS, **40**, 783, 1955; G. Gamow, these PROCEEDINGS, **41**, 7, 1955; J. R. Platt, these PROCEEDINGS, **41**, 181, 1955; D. P. Bloch, these PROCEEDINGS, **41**, 1058, 1955; C. Levinthal and H. R. Crane, these PROCEEDINGS, **42**, 436, 1956.

[9] C. Levinthal, these PROCEEDINGS, **42**, 394, 1956.
[10] D. Mazia and W. Plaut, *Biol. Bull.*, **109**, 335, 1955.

A METHOD FOR INCREASING BRAIN SEROTONIN WITHOUT INCURRING SOME OF THE PERIPHERAL EFFECTS OF THE HORMONE

BY D. W. WOOLLEY, E. VAN WINKLE, AND E. SHAW

ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, NEW YORK CITY

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The object of the present study was to determine whether the serotonin content of the brain could be markedly increased by peripheral medication, but without incurring the peripheral actions of this hormone (contractions of the intestines, increased blood pressure, etc.) at the same time. A further object was to determine whether the increased brain serotonin would then bring about behavioral changes in the animals—in other words, whether the excess hormone would still exert a central effect when some of the receptors in the periphery were blocked. The reasons for this study were the following.

In 1954 Woolley and Shaw[1, 2] presented evidence which indicated that certain mental disorders (e.g., schizophrenia) probably arose from a deficiency of serotonin in the brain. This deficiency was pictured as arising from a metabolic failure of the central nervous system to produce enough of this hormone. The nature of the evidence was such as to implicate serotonin in the etiology of the disease, but it did not unequivocally point to a deficiency rather than an excess of the hormone.[2] One of the purposes of the present investigation was to develop a method which might help distinguish between the alternate possibilities of too much or too little serotonin in the brain as the causative condition of the disorders.

The earlier work had shown[1, 3] that serotonin, although it was formed both in the brain and in the carcass of an animal, did not pass readily between these two parts. Serotonin injected peripherally could not be detected by chemical procedures in the brain, apparently because it did not pass the blood-brain barrier. As a result, there seemed little hope of attempting to raise the serotonin content of the brain by peripheral administration of the hormone. This difficulty was clearly stated in the original paper, and subsequent clinical trials have substantiated it. For example, the peripheral injection of serotonin into patients suffering from schizophrenia has not alleviated the disease. Even if the causative factor in the disorder is a deficiency of serotonin in the brain, the mere administration of the hormone in the periphery could hardly be expected to increase the content of the brain. Direct injection of the hormone into the central nervous system is too dangerous to be contemplated in human subjects. Consequently, a means of changing brain serotonin levels by peripheral medication must be sought if the fundamental hypothesis is to be tested.

A means of doing this has recently been found. Udenfriend *et al.*[4] have found that the normal precursor of serotonin, viz., 5-hydroxytryptophan, when injected peripherally into animals, passes the barrier and is converted into excess serotonin