QUANTITATIVE ASPECTS OF DEOXYRIBOSE NUCLEIC ACID (DNA) METABOLISM IN AN AMICRONUCLEATE STRAIN OF TETRAHYMENA [1]

BARBARA BROWN McDONALD [2, 3]

Department of Zoology, Columbia University, New York 27, N. Y.

Of extreme cytological and genetic interest is the heteronucleate condition—the presence of both micro- and macronuclei—in most ciliate protozoans. The micronucleus is a permanent cell organelle, normally diploid, which divides mitotically during vegetative cell growth, and meiotically during conjugation or autogamy. On the other hand, the macronucleus, which contains far more chromatin as indicated by its size and staining capacity, appears to pull apart amitotically during vegetative growth; during conjugation or autogamy, it degenerates. Following this phenomenon, the new macronucleus develops from a division product of the synkaryon, presumably as a result of endomitosis (as demonstrated by Grell, 1953a, in a suctorean, *Ephelota gemmipara*).

Such obvious differences between these two types of nuclei led to the earlier belief that they contained two types of chromatin—idiochromatin in the micronucleus, and trophochromatin in the macronucleus (as noted by Wichterman, 1953). By a cytochemical study of vegetative individuals of *Paramecium caudatum,* however, Moses (1950) found that, although the macronucleus contains perhaps 40 times as much nucleoprotein as the micronucleus, both types of nuclei contain very similar *relative* amounts of deoxyribose nucleic acid (DNA), ribose nucleic acid and protein, which would seem to indicate that both are metabolically active. More recently, however, a difference in protein composition has been reported by Alfert and Goldstein (1955) for another ciliate, *Tetrahymena pyriformis* (mating types I and II, Elliott). Using a recently developed, direct staining method for basic protein (Alfert and Geschwind, 1953), they found the ratio of basic protein: DNA in the micronucleus to be about 1.7 times greater than that in the macronucleus.

As a result of its large amount of chromatin, the macronucleus might be expected to have more genetic influence than the micronucleus. This has, indeed, been found by Sonneborn (1947) to be the case in *Paramecium aurelia.* Organisms with a micronucleus of one genotype and a macronucleus of another (resulting from regeneration, after conjugation, of a part of the old macronucleus) show the characteristics carried by the macronucleus. He has referred to the micronucleus as the germinal nucleus, and the macronucleus as the somatic nucleus. More recently, Sonneborn (1954) has clearly demonstrated that genes in the micro-

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

[2] During part of this investigation the author was supported by a fellowship from The McCallum Foundation.

[3] Present address: Department of Biology, Dickinson College, Carlisle, Pennsylvania.

nucleus may be completely inactive. Phenotypic expression following conjugation in ciliates is, in fact, most complex. In *Paramecium* and *Tetrahymena,* organisms of identical genotype may differ as to phenotype (for example, mating type—Sonneborn, 1939; Nanney and Caughey, 1953). In some cases, differentiation appears to be influenced by the cytoplasm, which of course had been influenced by the pre-conjugation macronucleus (Sonneborn, 1951, 1953; Nanney 1953a, 1956, 1957). It appears that location in the cytoplasm determines which division products of a synkaryon will be micro- and which will be macronuclei, in both *Paramecium* (Sonneborn, 1953) and *Tetrahymena* (Nanney, 1953b).

As exceptions to the rule that ciliates normally contain both types of nuclei, races and individuals occur in which micronuclei are absent. Such amicronucleate organisms, of course, cannot undergo autogamy, although they may conjugate with normal organisms (Chen, 1940; Sonneborn, 1953), and receive from them half a set of micronuclear chromosomes. Some amicronucleate strains are capable of vegetative growth for extended periods of time. Experimental strains of *Tetrahymena pyriformis,* for the most part, have no micronuclei (Corliss, 1953). In contrast, amacronucleate strains of ciliates have not been reported. Seshachar and Dass (1953) have observed in a peritrich, *Epistylis articulata,* that some daughter cells, receiving no macronucleus but only a micronucleus as a result of abnormal fission, eventually regenerated a macronucleus from a division product of the micronucleus. These various circumstances indicate that whereas the macronucleus is essential to the organism, the micronucleus is not. In amicronucleate strains, the macronucleus rather than the micronucleus is necessarily a permanent cell organelle.

In the present experiments, quantitative aspects of DNA metabolism in the macronucleus have been investigated in an amicronucleate strain of *Tetrahymena pyriformis*—strain H, isolated by Hetherington in 1930 (1933). Having no micronucleus, this strain has not been observed to undergo conjugation or autogamy, with the attendant degeneration and subsequent reformation of the macronucleus.

By means of microspectrophotometric analysis, the amount of DNA (indicated by the uptake of Feulgen dye, which has been shown to be proportional to the total DNA—Swift, 1953) has been determined at different stages of growth. The answers to a number of questions have been sought. For example, in the apparent absence of a mitotic apparatus, does the macronucleus divide equally between daughter cells? Before the next division, is the DNA precisely duplicated, as in mitotically dividing cells (Swift, 1950), or does an indefinite amount of synthesis occur? Does the DNA remain stationary in cells of a mass culture which has reached maximum growth? Finally, during what part of the growth cycle does the macronucleus synthesize DNA?

Methods

I. *Stock cultures*

The stock of *Tetrahymena pyriformis* H used in these experiments was obtained from Mr. Sheldon Greer of the Department of Zoology, Columbia University, who in turn had obtained it from Dr. Seymour Hutner of the Haskins Laboratories, New York. The cells were cultured in 5-ml. amounts of 2% proteose peptone broth (Difco) in 16 × 150 mm. Pyrex test tubes. The tubes were arranged in a slanting position in a 25° C. constant temperature room. Stock cultures were

transferred weekly (0.1-ml. inocula), at which time samples were spread on proteose peptone agar to test for possible contamination.

Cultures for experimental purposes were inoculated on the day before they were to be used, to insure that the cells would be in the logarithmic stage of growth.

II. *Fixation and staining of mass cultures*

Cells from a culture growing in a test tube were fixed directly on a chemically clean microscope slide, which was first placed in a paraffin-coated paper box (80 mm. long, 26 mm. wide, 12 mm. deep). After 10 ml. fixative (9 parts absolute alcohol: 1 part glacial acetic acid) were added, 0.25-0.5 ml. of the culture was delivered slowly from a fine-tipped pipette over the slide. In 10 minutes the slide was removed, drained, and held on its side against a paper towel while absolute alcohol was delivered slowly along the other edge from a fine-tipped pipette. The slide was then placed in absolute alcohol. After 10 minutes or longer, the slide was drained, 0.5% celloidin was run over the surface to which the cells were attached, and the slide was again drained and placed in 80% alcohol (modified from Chen, 1944). Throughout the subsequent treatment of the slide, precautions were taken so that the celloidin would not become dry and thus loosened from the slide.

Nuclei were stained according to the Feulgen procedure essentially as described by Di Stefano (1948) except that they were hydrolyzed in 2 N hydrochloric acid at 40° C. instead of in 1 N hydrochloric acid at 60°. This modification was used so that the time of hydrolysis would be less critical, since cells on different slides were to be compared. Photometric measurements indicated 55 minutes to be the optimal hydrolysis time.

After treatment with Feulgen preparation and bleach, the slide was rinsed during 5 minutes in 5 changes of water. The slide was run up through the alcohols to 1 part ether: 1 part absolute alcohol (which removed the celloidin), through absolute alcohol and xylol, and finally was mounted in oil of refractive index 1.572, which closely matched the refractive index of the cells.

III. *Growth of individual cells*

In a set of experiments to determine the generation times in small clones, individual cells were isolated under sterile conditions to hanging drops of proteose peptone broth. Growth was followed by observations every 15 minutes through a dissecting microscope.

Although the hanging drop method was quite satisfactory for following growth, it was not practical if the cells were to be fixed; when the cover slip was removed, the small drop of broth dried up very quickly. A different method was used to grow the cells in larger drops of medium (about 0.02 ml.) in small watch glasses (U. S. Bureau of Plant Industry Model, A. H. Thomas). These dishes (coated with silicone from Dow-Corning Sight Savers before sterilization to prevent the drops from spreading) were kept in the moist chamber of a petri plate lined top and bottom with wet filter paper in which holes had been cut for viewing the drops. To prevent fogging of the inner surface of the petri top, it was treated with a paste (Clersite, Chicago, Ill.) applied with sterile Wipettes.

For convenience, individual cultures were grown in the laboratory, at temperatures varying from 24–27°, averaging 25° C.

IV. *Fixation and staining of individual cells*

Individual containers for the fixation of cells from small clones were prepared by sealing paraffin rings to slides. The rings were formed by dipping a silicone-coated vial (10 mm. outside diameter), filled with cold water, into molten paraffin, and then into cold water. The paraffin coat was cut into rings about 5 mm. deep, which were slipped off the vial and sealed to the slide by careful heating. Two

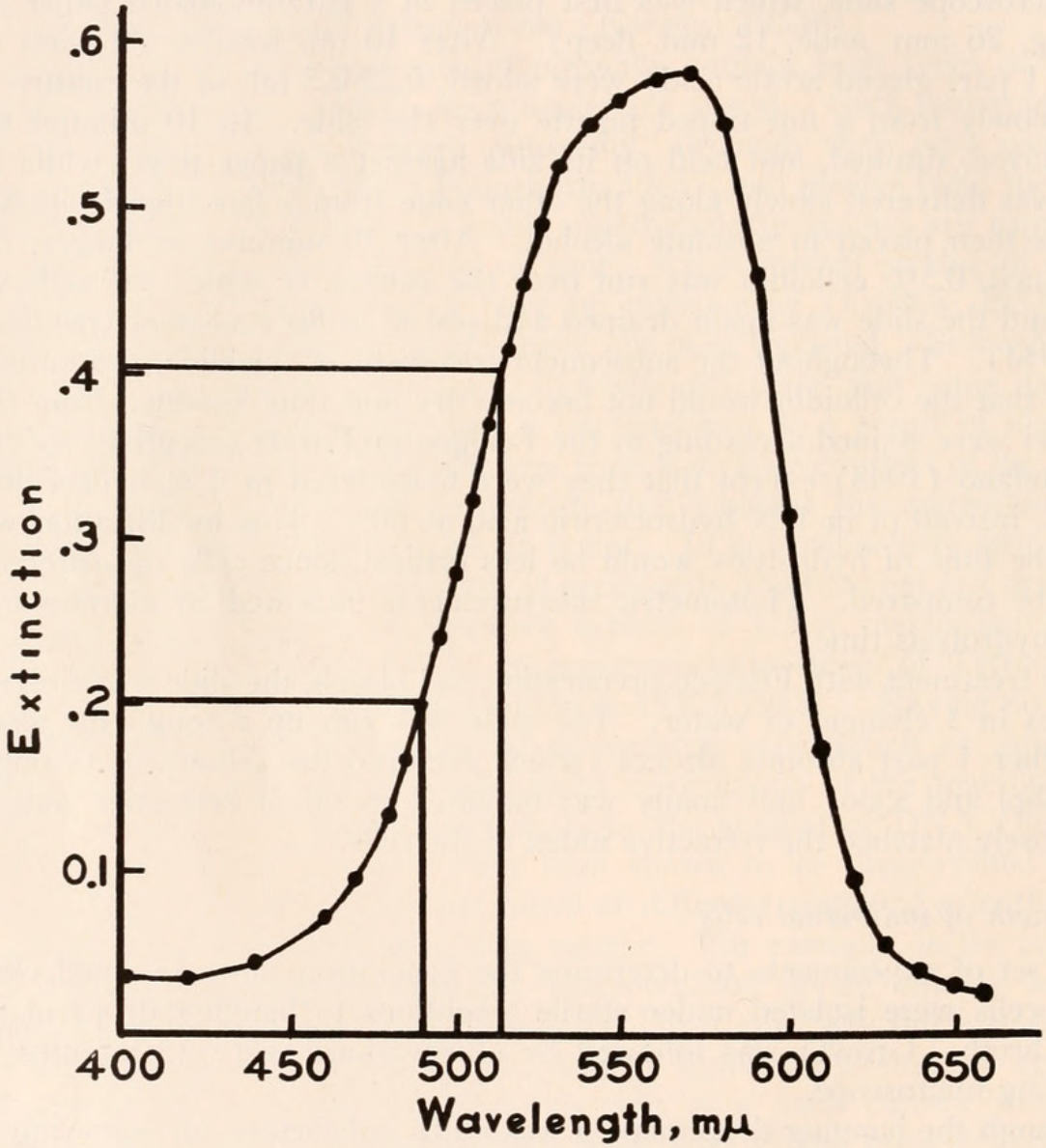

FIGURE 1. Absorption curve of a *Tetrahymena* macronucleus stained with Feulgen dye. The two wave-lengths (490 mμ and 514 mμ) used in making photometric measurements are indicated.

were placed on each slide, directly above notched circles which had been drawn on the opposite side of the slide with a diamond pencil. The notches, facilitating the location of cells, could be seen at the inner edges of the paraffin rings.

Fixation was carried out under two dissecting microscopes—one for the isolation of the cell, and one for its fixation. About 0.4 ml. of fixative was placed in one of the paraffin containers. The cell was delivered carefully from a micropipette at the surface of the slide, and a coverslip was placed on top of the con-

tainer to minimize evaporation which would cause agitation of the fixative. After 10 minutes the fixative was removed with a pipette (whose fine tip was drawn out at an angle), and absolute alcohol was then carefully added. Usually this procedure resulted in sticking the cell to the slide; occasionally the cell became loose, in which case the alcohol was removed, and fresh alcohol was added. In this way the majority of cells could be saved.

After cells had been fixed in both containers on a slide, notations were made of the cells' locations and the slide was placed in a petri plate containing absolute alcohol. Within an hour the paraffin rings became loosened and were removed with a forceps. At this time the presence of the cells was checked under a dissecting microscope. The slide was rinsed in xylol-alcohol to remove any adhering paraffin molecules, in absolute alcohol, and then celloidin was run over its surface

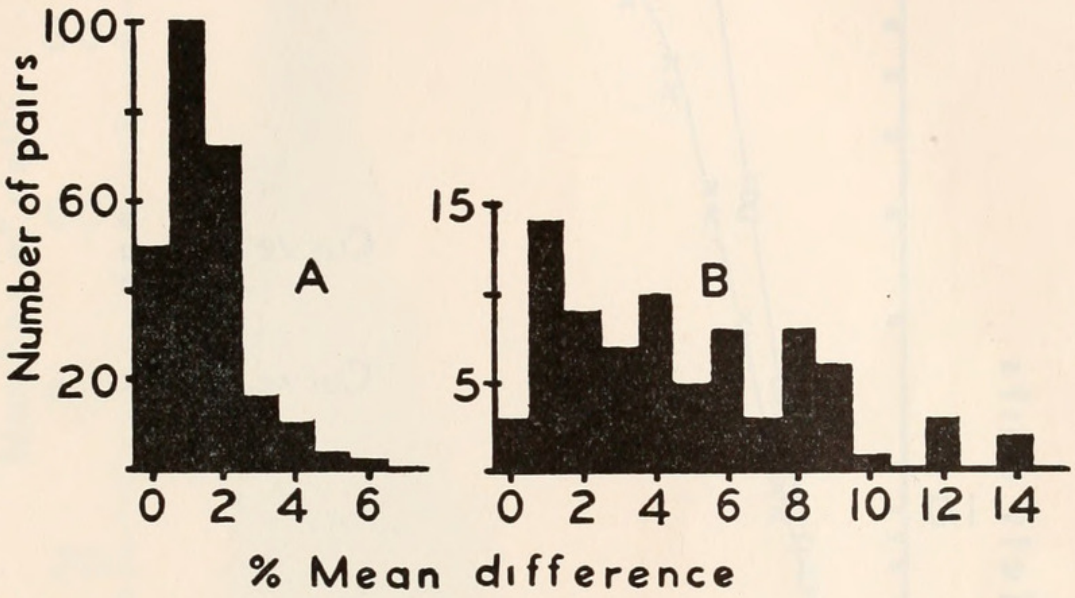

FIGURE 2. Variability of duplicate DNA determinations compared with variability of DNA content in sister cells. (A) % Mean difference in 263 pairs of duplicate DNA determinations. Range 0.0 to 5.7%, mean 1.4%. (B) % Mean difference of DNA values in 83 pairs of sister cells. Range 0.1 to 14.2%, mean 4.7%.

as previously described. Subsequent treatment of the slide differed from that already described only in that the celloidin was removed, after staining, by placing the slide in a petri plate containing ether-alcohol, when the presence of the cells was again checked under the microscope.

V. *Photometric methods*

Measurements were made with the microspectrophotometric apparatus described by Pollister (1952), using a Bausch & Lomb monochromator. Because it was desired to measure all the small clones fixed, and many of the nuclei were not spherical, the two-wave-length method described by Ornstein (1952) and Patau (1952) was used. Figure 1 shows a typical absorption curve for a Feulgen-stained nucleus. The two wave-lengths selected were 490 mμ and 514 mμ (which were also used by Patau). For a single DNA determination, two readings through both nucleus and background were made at each wave-length. Two determina-

tions were made and averaged for each cell from a small clone; between determinations, the fine adjustment and the condenser of the microscope were refocused, and the phototube diaphragm was readjusted. The % mean difference of duplicate determinations on 263 cells ranged from 0 to 5.7, with an average of 1.4 (Fig. 2A).

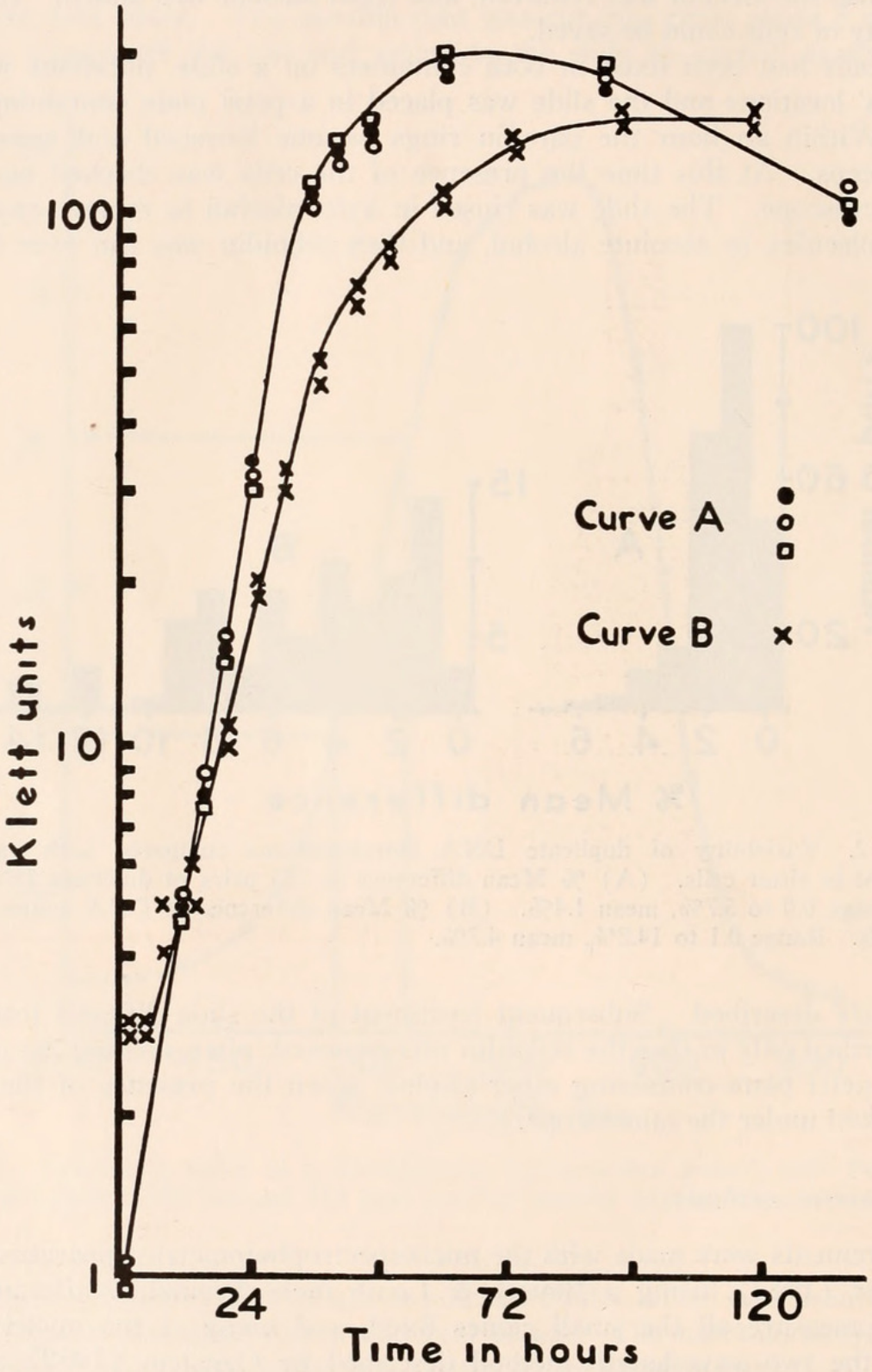

FIGURE 3. Growth curves for *Tetrahymena pyriformis* H. Both curves are drawn from turbidity readings in a Klett photoelectric colorimeter. Curve A is based on three tubes of cells grown in a roller. Curve B is based on a series of tubes, slanted but not otherwise aerated. Maximum growth for both A and B was about 3×10^5 cells per ml. (Although all the growth tubes were the same size, two different sizes of tubes were used for the two sets of readings, accounting for the different heights of the curves.)

I. *Growth of Tetrahymena*

The ability of *Tetrahymena* to thrive under controlled conditions in non-particulate, sterile medium makes it an ideal protozoan for biological research. Although proteose peptone broth was used in this investigation, *Tetrahymena* can be grown also in media of carefully measured, known constituents (Elliott and Hayes, 1953; Kidder, Dewey and Heinrich, 1954). The growth response in both proteose peptone and defined media is quite reproducible.

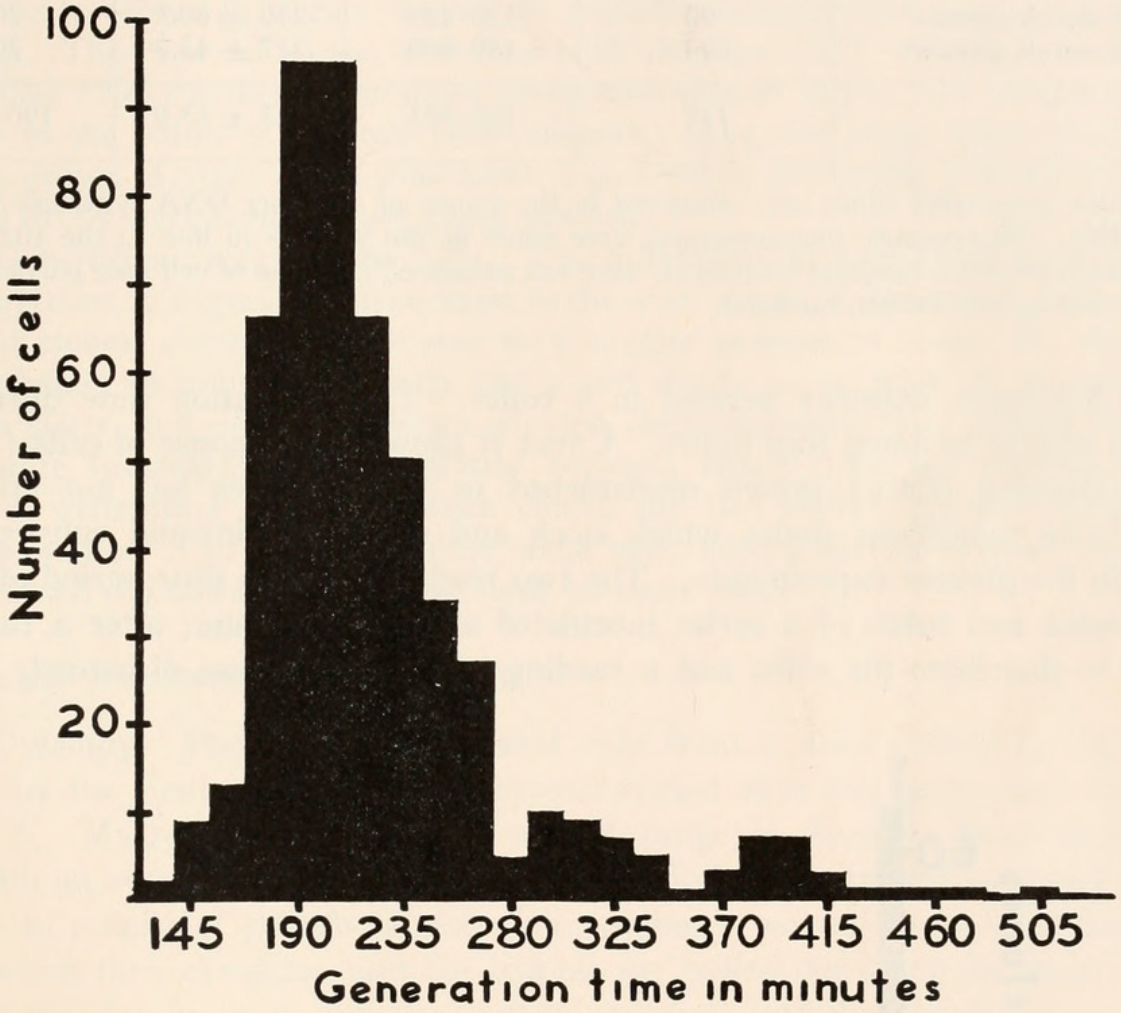

FIGURE 4. Generation times in isolated clones. A total of 515 cells (160 two-cell clones and 195 individual cells) are represented. Data for the different groups of cells included in this histogram are given in Table I.

A. *Growth in mass cultures.* *Tetrahymena* shows a pattern of growth similar to that for other micro-organisms—a short lag period (when the inoculum is from a culture in stationary phase); a logarithmic stage of growth, during which the cell number doubles in regular time intervals; a period of deceleration, when the rate of cell divisions decreases; and a stationary period, during which the number of cells remains constant, with few cell divisions or deaths.

In Figure 3 are shown two different growth curves for cells grown in 2% proteose peptone broth, based on turbidity readings in a Klett photoelectric colorimeter. Curve A represents the growth response of three tubes of cells (inocula

TABLE I

Generation times of individual cells

Growth conditions	Number of cells	Range (min.)	Mean ± S.D. (min.)	Mode (min.)
Hanging drops	170 (includes 84 2-cell clones)	135–505	215 ± 53.7	190–205
Drops in watch glasses	152 (76 2-cell clones)	150–465	220 ± 58.1	190
Drops in watch glasses*	90	155–480	240 ± 60.2	205
Drops in watch glasses*	103	150–400	227 ± 43.7	205
Total	515	135–505	223 ± 53.0	190–205

* These generation times were observed in the course of studying DNA synthesis (see Results, IIB3). Photometric measurements were made of the 90 cells in line 3; the 103 cells in line 4, which received identical treatment, were not measured (because of cell loss, particles overlying a nucleus, or a broken nucleus).

from a log phase culture) aerated in a roller. The generation time during log phase is seen to be about four hours. Curve B shows the response of cells (inocula from stationary phase) grown undisturbed in slanted tubes but not otherwise aerated—the conditions under which stock and mass experimental cultures were grown in the present experiments. The two readings at each time period of Curve B represent two tubes of a series inoculated at the same time; after a tube was shaken to distribute the cells, and a reading was made, it was discarded. Curve

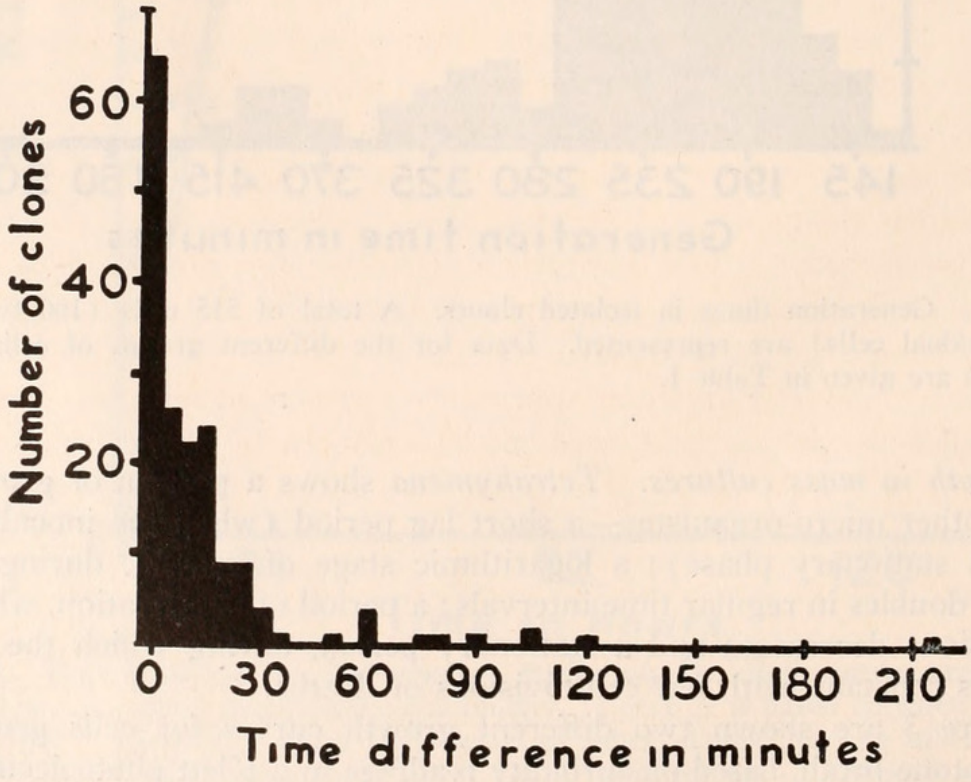

FIGURE 5. Differences in generation times between sisters in 174 two-cell clones.

B differs noticeably from Curve A in the long generation time of 7 hours and the extended period of deceleration. These conditions undoubtedly resulted from the limited availability of oxygen. Both curves clearly indicate the reproducibility of growth response of *Tetrahymena* in 2% proteose peptone broth under controlled conditions.

B. *Growth in small clones.* Single cells which had been isolated from cultures in log phase to either hanging drops or drops in small watch glasses were timed through two divisions. Generations were calculated between the times the sisters actually separated, to the nearest 5-minute intervals. The distribution of generation times is shown in Figure 4, and the separate experiments are summarized in Table I. The over-all range is 2 hours 15 minutes (135 minutes) to 8 hours 25 minutes (505 minutes); the mean generation time is 223 ± 53.0 minutes. This is close to the value of 4 hours (240 minutes) computed from Klett readings of cultures grown in tubes which were aerated on a roller, indicating that the conditions in small drops were favorable for rapid growth.

C. *Generation times of sister cells.* The differences in generation times of sister cells are shown in Figure 5. In contrast to the wide range of generation times among different clones, sisters tend to have very similar generation times, the mean difference being 14 minutes. Rarely did a cell divide more than 30 minutes later than its sister, and often the sisters divided simultaneously (*i.e.*, within the same two-minute interval). The difference between sisters was not correlated with length of generation time. In some clones the two sisters divided almost synchronously after 5 to 7 hours, while some cells with short generation times had sisters which did not divide until an hour or more later.[4]

II. *Nuclear phenomena in the life cycle*

A. *Cytology.* Photographs of several cells from a mass culture in log phase, stained by the Feulgen method and counterstained with fast green, are shown in Figure 6. Macronuclei of *T. pyriformis* H range in diameter from about 5 to 12 μ, with an average size of about 8 μ. Before division starts, the enlarged nucleus appears to consist of strands of chromatin granules wound into a ball (stage 1). The nucleus then elongates, and the strands are pulled out along its length (stage 2). As division proceeds, and the nucleus separates into the two daughters (stage 3), broken ends of strands can sometimes be seen. Part of a strand (or strands) may remain behind in the cytoplasm (stage 4) where it condenses into a small Feulgen-positive body. Similar in appearance to micronuclei, these bodies are not present in all cells, and in time seem to be resorbed by the cytoplasm. One of these particles is visible in the left of the two interphase cells (stage 5). The relatively homogeneous interphase nucleus appears to be made up of fine threads of chromatin.

[4] Separation of sisters and "conditioning" of medium did not seem to affect generation time. About 12 hours after 9 individuals had been isolated to drops of broth in watch glasses, all but one of the cells in each drop resulting from the interim divisions were removed. An hour after these cells had divided, one sister of each pair was placed in a fresh drop of medium. All the pairs of separated sisters divided within 20 minutes of each other—three divided synchronously, five cells in fresh medium divided sooner than their sisters, and one divided later.

In these photographs, cytoplasmic vacuoles are demonstrated by the fast green stain, but the mouths of the organisms are not clearly distinguishable.

B. *Photometric analysis of DNA.* 1. *Amount of DNA per cell in mass cultures.* A sample of cells from a logarithmically growing culture (I) (24 hours after inocu-

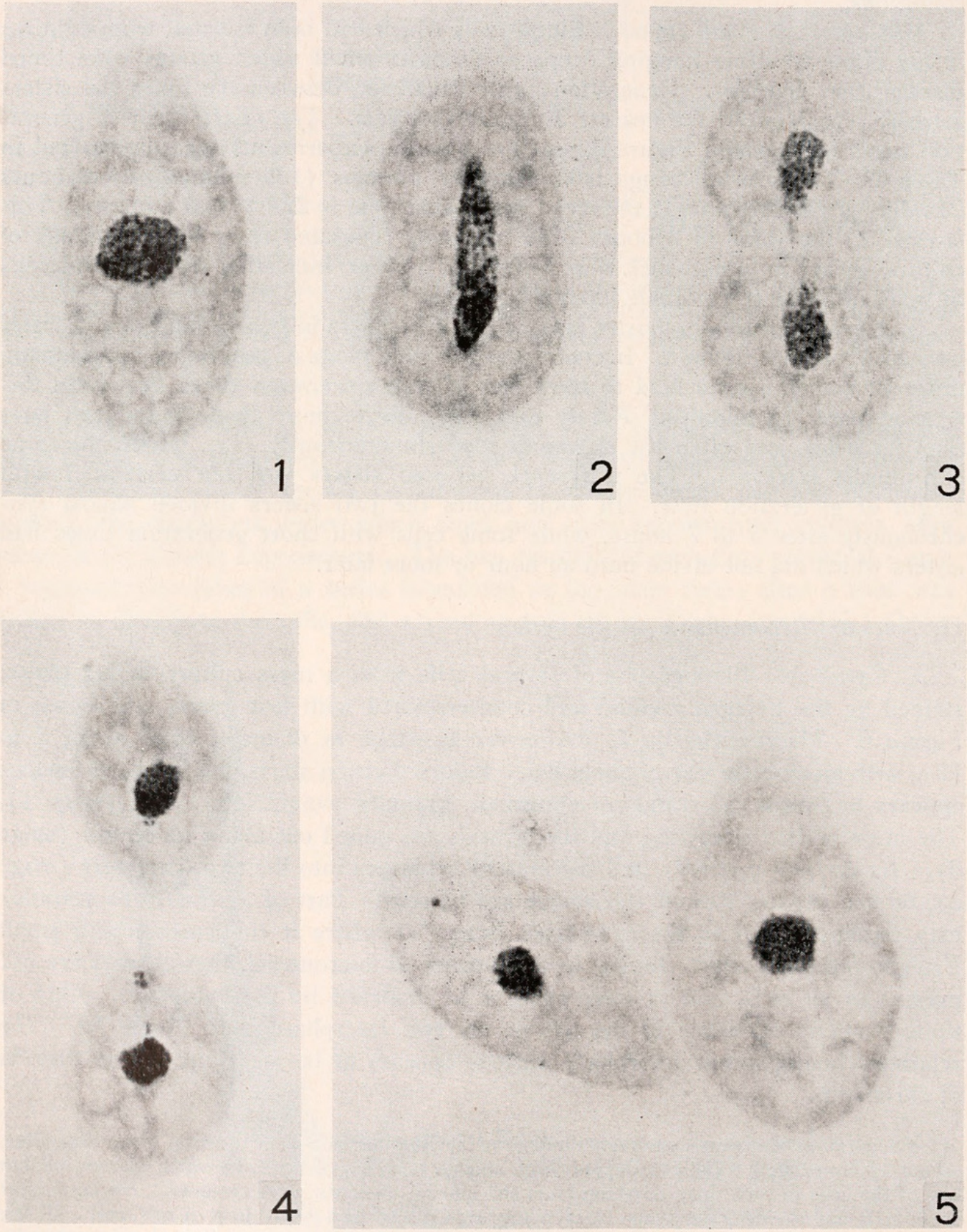

FIGURE 6. Photographs of *Tetrahymena pyriformis* H stained with Feulgen and fast green dyes. Dividing cells in different stages are shown in 1, 2, 3, and 4, and interphase cells in 5. See text for further explanation. Magnification about 1050 ×.

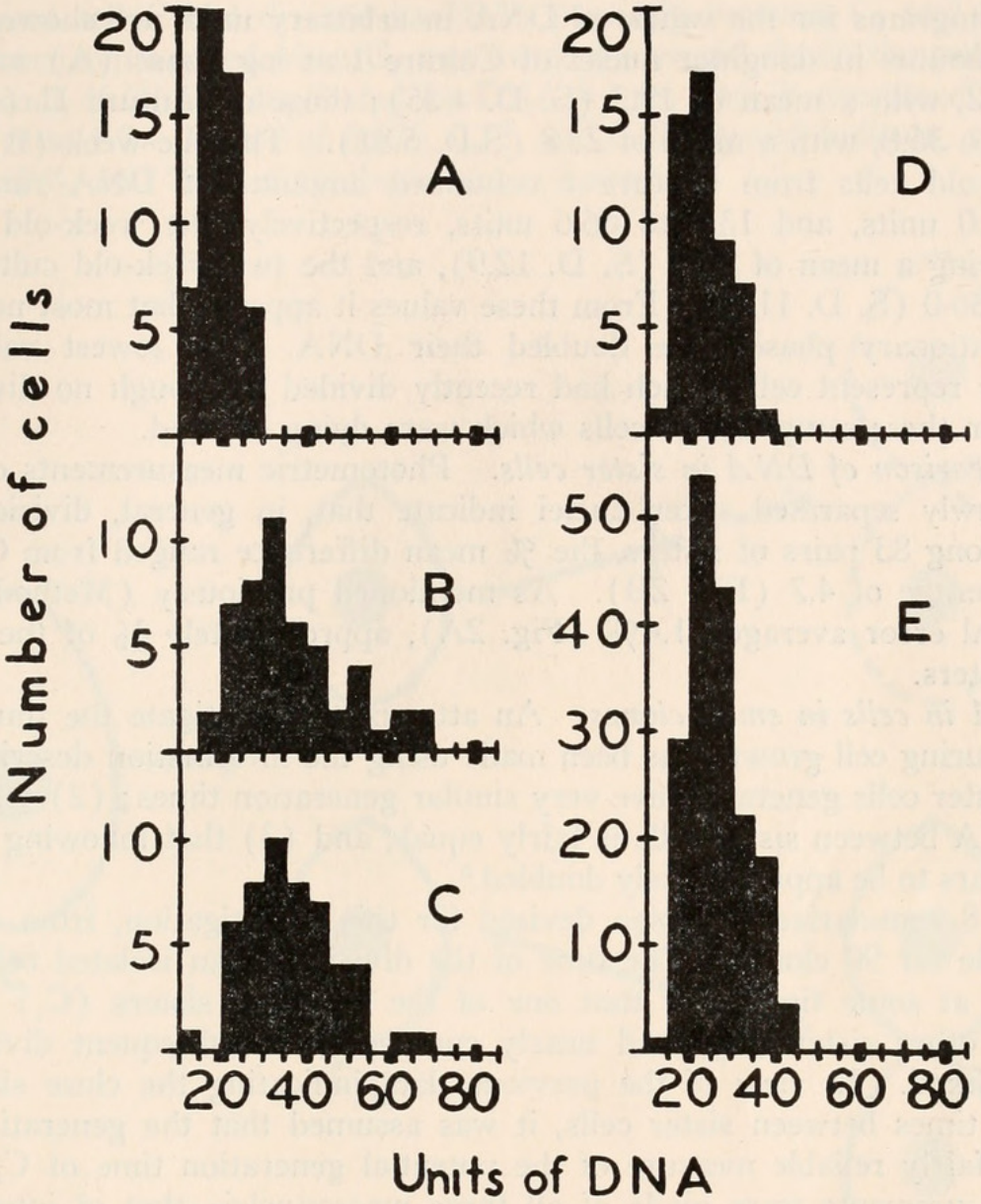

FIGURE 7. Distribution of DNA in mass cultures and isolated cells. (A) Culture I, one day old (log phase). Amount of DNA in 50 dividing daughters (each from a different pair). Range 12.5 to 29.2; mean 19.3 ± 4.35 S. D.; mode 17. (B) Culture I, one week old (stationary phase). Amount of DNA in 50 cells. Range 17.9 to 68.0; mean 35.9 ± 12.9 S. D.; mode 32. (C) Culture I, two weeks old. Amount of DNA in 50 cells. Range 13.9 to 66.6; mean 36.0 ± 11.0 S. D.; mode 32. (D) Culture II, one day old (log phase) (subculture of one-week old Culture I). Amount of DNA in 50 dividing daughters (each from a different pair). Range 13.7 to 36.8; mean 22.8 ± 5.81 S. D.; mode 22. (E) Isolated dividing daughters. Amount of DNA in 173 cells (83 pairs and 7 individuals). Range 12.9 to 43.2; mean 25.7 ± 6.15 S. D.; mode 22.

lation) was fixed and stained. One daughter nucleus of each of 50 dividing pairs (similar to stage 4, Fig. 6) was measured; presumably these values would indicate the basic, or minimal, amount of DNA. (Since the cytoplasm was invisible, criteria for dividing daughters were shape and appearance of nuclei—two rather tear-shaped and granular-looking nuclei, usually with part of a strand of chromatin extending from one toward the other.) One week later this culture, in stationary phase, was sampled again, and 50 nuclei were measured. At the same time, a subculture (II) was inoculated from Culture I. Twenty-four hours later Culture II was sampled, and 50 of its dividing daughters were measured. When Culture I was two weeks old it was sampled again, and 50 more nuclei were measured.

The histograms for the values of DNA in arbitrary units are shown in Figure 7. The amounts in daughter nuclei of Culture I at log phase (A) ranged from 12.5 to 29.2, with a mean of 19.3 (S. D. 4.35); those of Culture II (D) ranged from 13.7 to 36.8, with a mean of 22.8 (S.D. 5.81). The one-week-(B) and two-week-(C) old cells from Culture I contained amounts of DNA ranging from 17.9 to 68.0 units, and 13.9 to 66.6 units, respectively, the week-old stationary culture having a mean of 35.9 (S. D. 12.9), and the two-week-old culture having a mean of 36.0 (S. D. 11.0). From these values it appears that most non-dividing cells in stationary phase have doubled their DNA. The lowest values might conceivably represent cells which had recently divided (although no dividing cells were seen in these samples), or cells which were dying or dead.

2. *Comparison of DNA in sister cells.* Photometric measurements of DNA in pairs of newly separated sister nuclei indicate that, in general, division is quite even. Among 83 pairs of sisters, the % mean difference ranged from 0.1 to 14.2, with an average of 4.7 (Fig. 2B). As mentioned previously (Methods, V), the instrumental error averaged 1.4% (Fig. 2A), approximately ⅓ of the difference between sisters.

3. *DNA in cells in small clones.* An attempt to investigate the time of DNA synthesis during cell growth has been made using the information described above: (1) that sister cells generally have very similar generation times; (2) that distribution of DNA between sister cells is fairly equal; and (3) that following a division, DNA appears to be approximately doubled.[5]

Figure 8 summarizes the plan devised for this investigation, from which data are available for 90 clones. The time of the division of an isolated cell (C) was noted, and at some time after that one of the resulting sisters (C_1) was fixed. When the other sister (C_2) had nearly completed its subsequent division it, in turn, was fixed. In view of the previous data indicating the close similarity of generation times between sister cells, it was assumed that the generation time of C_2 gave a fairly reliable measure of the potential generation time of C_1.[6] Photometric measurements were made of all three macronuclei—that of interphase cell C_1, and those of the two daughters of C_2 (designated C_{2a} and C_{2b}). Adding the DNA in C_{2a} and C_{2b} for the total amount in C_2 indicated the amount of DNA which C_1 would have contained by the time it reached its next division. The ratio of C_1/C_2, then, indicates the relative amount of DNA in C_1 when it was fixed.

[5] Some additional evidence suggesting the duplication of DNA comes from seven pairs of synchronously dividing sisters, each of whose four daughter nuclei were measured photometrically. Among four of these pairs, the two daughter mates with the lower total amount of DNA had 94%, 96%, 97%, and 97% as much DNA as the two higher daughter mates. In the other three pairs, the daughters had become detached, so the mates could not be differentiated. The most extreme possible relative values would have been 82%, 89%, and 96%; the closest possible, 91%, 99%, and 100%.

[6] The generation time of C_2 was known quite closely, although, since dividing cells were fixed, the time of separation had to be estimated by appearance. Cells which are in the process of dividing become quiescent until the division is nearly completed, when they begin swimming, pulling, and twisting. Assuming the time spent dividing to last from the time a cell quiets down and a constriction begins to appear, to the time the daughters actually pull apart, it has been observed to take from about 20 to 35 minutes, regardless of the length of generation time. In these experiments, an average of 25 minutes was used as a basis for calculation. For these cells, the length of interphase time, then, is based on the generation time (known time of separation at the first division to the estimated time of separation at the second division) minus 25 minutes.

If the original cell C had divided its DNA precisely between C_1 and C_2, and no DNA had been synthesized when C_1 was fixed, one would obtain the ratio 0.5. On the other hand, if C_1 had duplicated its DNA by the time it was fixed, one would obtain the ratio 1.0. Ratios of 0.625, 0.75, and 0.875 would indicate, respectively, that 25%, 50%, and 75% of synthesis had occurred.

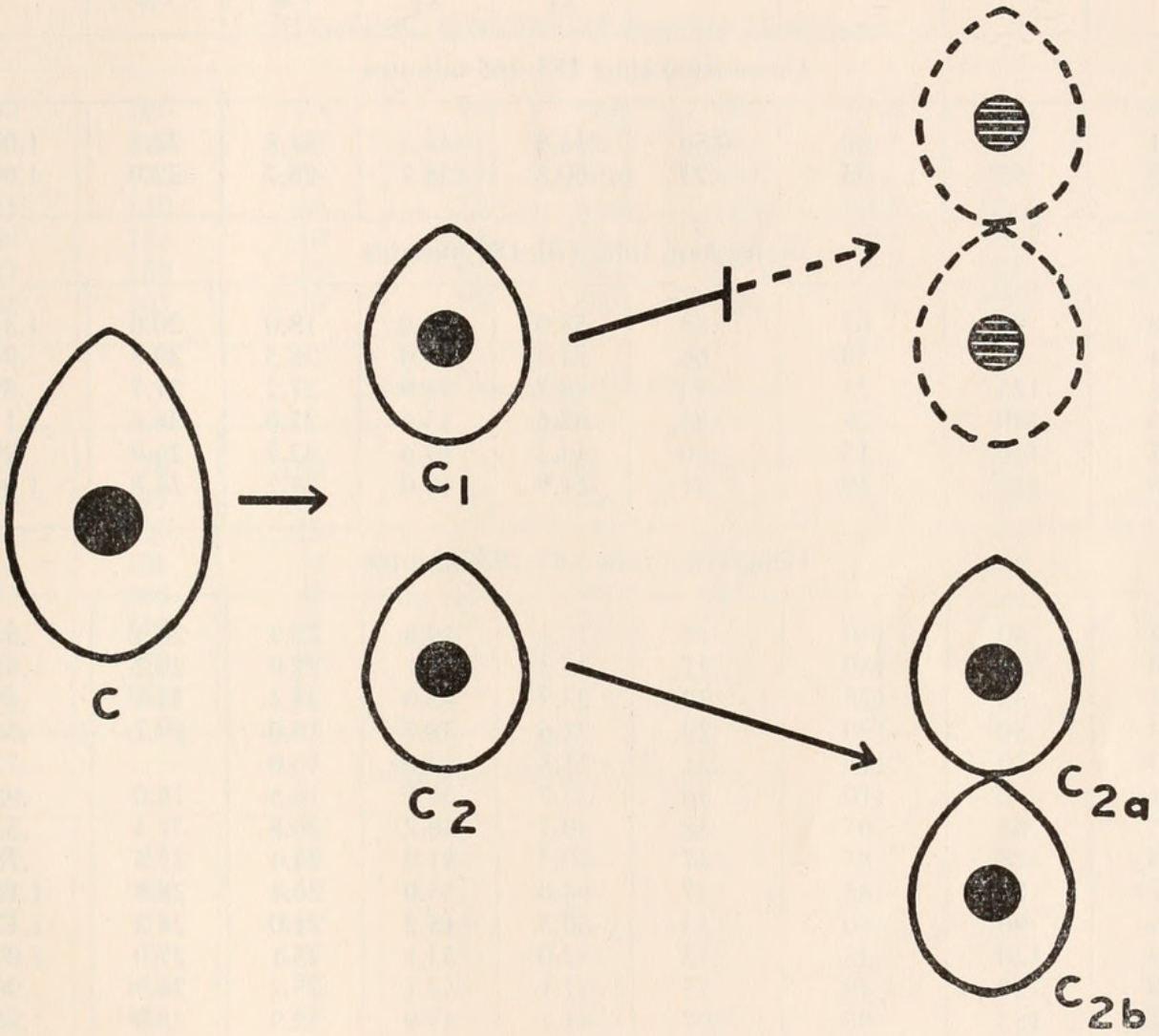

FIGURE 8. Scheme for fixing sister cells. The figure on the left represents an isolated cell (C), in time dividing into the two sister cells in the middle (C_1 and C_2). During the following interphase C_1 was fixed, as indicated by the vertical line, when its DNA might or might not have increased. C_2 was not fixed until its next division, as indicated at bottom right; its generation time was a measure of the potential generation time of its fixed sister, C_1. The total DNA in the C_2 daughters (C_{2a} and C_{2b}) represented the amount which C_1 would have contained at its next division.

In Table II the data are summarized, with the 90 clones grouped according to generation times, and each group arranged according to the age of C_1 when it was fixed. Also included are the period of interphase (see footnote 6) elapsing between the fixation of C_1 and the start of the C_2 division, the relative interphase age of C_1 (age of C_1/length of C_2 interphase), the amounts of DNA in the measured cells, and the relative DNA content of C_1 (C_1 DNA/C_2 DNA). Table I, line 3, summarizes the generation time data for the C_2 cells, and Figure 7E shows the distribution of DNA values for C_{2a} and C_{2b}.

TABLE II

Photometrically measured clones arranged according to generation times

Clone No.	Age C_1 (min.)	Time to next div. (min.)	Age C_1 / Interphase*	Amount of DNA (in arbitrary units)				C_1 DNA / C_2 DNA
				C_1	C_2	C_{2a}	C_{2b}	
Generation time 155–165 minutes								
1	75	60	0.56	44.8	44.3	21.8	22.5	1.01
2	95	35	.73	60.8	58.7	26.7	32.0	1.04
Generation time 170–180 minutes								
3	90	65	.58	53.0	38.6	18.0	20.6	1.37‡
4	95	50	.66	54.3	58.0	28.5	29.5	.94
5	125	25	.83	48.7	54.9	27.2	27.7	.89
6	130	25	.84	62.6	53.6	25.0	28.6	1.17
7	140	15	.90	49.2	49.6	22.7	26.9	.99
8	145	10	.94	51.9	49.0	24.2	24.8	1.06
Generation time 185–195 minutes								
9	30	140	.18	27.1	49.8	23.7	26.1	.54
10	35	130	.21	35.3	57.1	27.9	29.2	.62
11	35	125	.22	23.2	48.0	23.4	24.6	.49
12	50	120	.29	21.6	38.7	19.0	19.7	.56
13	50	110	.31	24.5	32.0†	16.0	—	.77
14	60	110	.35	33.7	34.5	16.5	18.0	.98§
15	65	105	.38	30.7	58.2	26.8	31.4	.53
16	75	85	.47	40.2	51.9	24.4	27.5	.78
17	75	85	.47	64.0	55.0	26.1	28.9	1.16
18	90	80	.53	50.5	45.2	21.0	24.2	1.12
19	120	45	.73	52.0	52.1	25.1	27.0	1.00
20	120	40	.75	47.1	52.1	25.2	26.9	.90
21	155	05	.97	41.4	47.9	22.9	25.0	.86
Generation time 200–210 minutes								
22	30	150	.17	32.1	60.0	29.9	30.1	.53
23	35	150	.19	26.6	43.7	20.6	23.1	.61
24	35	145	.20	27.8	43.4	21.6	21.8	.64
25	45	130	.26	28.0	35.5	17.7	17.8	.79§
26	65	115	.36	33.3	46.8	23.0	23.8	.71
27	90	90	.50	56.6	58.7	29.0	29.7	.97
28	90	85	.51	34.8	48.2†	24.1	—	.72
29	90	85	.51	32.1	40.6	19.6	21.0	.78
30	90	85	.51	36.8	37.3	18.1	19.2	.99
31	95	85	.53	42.6	78.6	38.8	39.8	.54§

* Interphase equals generation time minus 25 minutes.

† Estimate of C_2 DNA based on measurement of one daughter; other daughter unmeasurable.

‡ Unequal division of C obvious from C_1/C_2 ratio.

§ Unequal division of C presumed from exceptional amount of DNA in C_2, and unusual C_1/C_2 ratio for age of C_1.

TABLE II—*Continued*

Clone No.	Age C_1 (min.)	Time to next div. (min.)	Age C_1 / Interphase*	Amount of DNA (in arbitrary units)				C_1 DNA / C_2 DNA
				C_1	C_2	C_{2a}	C_{2b}	
Generation time 200–210 minutes—*Continued*								
32	100	85	.54	34.1	78.9	35.5	43.4	.43§
33	110	70	.61	60.9	63.6	31.5	32.1	.96
34	125	55	.69	53.8	62.6	31.1	31.5	.86
35	130	45	.74	48.8	49.3	23.8	25.5	.99
36	155	20	.89	34.2	33.1	15.7	17.4	1.08
37	160	15	.92	47.5	43.1	19.9	23.2	1.10
38	165	20	.89	40.4	34.4	16.4	18.0	1.17
Generation time 215–225 minutes								
39	70	130	.35	35.8	46.8	23.2	23.6	.77
40	95	100	.49	37.8	61.1	30.0	31.1	.62
41	150	45	.77	42.9	41.6	20.6	21.0	1.03
42	165	30	.85	49.7	47.3	21.7	25.6	1.10
43	165	25	.87	65.7	72.5	35.2	37.3	.91
44	170	30	.85	60.8	71.0	34.8	36.2	.86
45	180	10	.95	33.9	38.4	17.5	20.9	.88
46	185	15	.93	51.1	50.6†	25.3	—	1.01
Generation time 230–240 minutes								
47	35	180	.16	22.3	45.8	22.0	23.8	.49
48	60	150	.29	22.0	59.8†	29.9	—	.37‡
49	65	140	.32	35.6	54.4	25.6	28.8	.66
50	70	135	.34	45.2	40.5	19.4	21.1	1.12
51	140	70	.67	54.7	60.5	27.8	32.7	.91
52	155	55	.74	45.7	45.9	21.1	24.8	1.00
53	180	35	.84	62.7	59.4	29.2	30.2	1.05
54	175	30	.85	59.6	69.6	34.3	35.3	.86
Generation time 245–255 minutes								
55	30	190	.14	19.4	58.9	28.6	30.3	.34‡
56	35	195	.15	24.9	41.7	19.1	22.6	.60
57	60	160	.27	17.6	27.5	12.9	14.6	.64
58	130	90	.57	48.5	64.4	27.6	36.8	.75
59	145	85	.63	41.6	51.6†	25.8	—	.81
60	150	75	.67	43.8	43.8	21.0	22.8	1.00
61	155	65	.71	41.0	37.0	17.5	19.5	1.11
62	160	65	.71	45.7	50.4	25.1	25.3	.91
63	160	60	.73	36.4	47.7	23.5	24.2	.76
64	175	55	.76	46.2	47.8	22.5	25.3	.98
65	190	30	.86	41.0	34.7	17.2	17.5	1.18‡

TABLE II—*Continued*

Clone No.	Age C_1 (min.)	Time to next div. (min.)	Age C_1 / Interphase*	Amount of DNA (in arbitrary units)				C_1 DNA / C_2 DNA
				C_1	C_2	C_{2a}	C_{2b}	
Generation time 260–270 minutes								
66	115	130	.47	29.4	75.5	33.4	42.1	.39‡
67	120	115	.51	41.8	65.1	32.3	32.8	.64
68	145	90	.62	46.8	61.0	29.6	31.4	.77
69	150	85	.64	56.1	65.2	28.7	36.5	.86
70	150	85	.64	47.2	43.8†	21.9	—	1.08
71	155	90	.63	33.2	40.2	18.3	21.9	.83
72	155	80	.66	35.0	34.9	16.1	18.8	1.00
73	200	40	.83	50.0	58.9	28.4	30.5	.85
Generation time 275–285 minutes								
74	65	190	.26	35.3	52.8	24.9	27.9	.66
75	65	185	.26	41.4	69.7	34.8	34.9	.60
76	200	55	.79	42.1	52.0	22.8	29.2	.81
Generation time 290 minutes								
77	120	145	.45	40.0	82.0	38.8	43.2	.49§
Generation time 315 minutes								
78	220	70	.76	69.9	64.2†	32.1	—	1.09
Generation time 320–330 minutes								
79	120	180	.40	30.8	43.7	20.5	23.2	.71
80	135	170	.44	45.0	76.5	37.2	39.3	.59§
81	180	120	.60	48.3	47.6	23.2	24.4	1.01
Generation time 335–345 minutes								
82	150	165	.48	27.2	54.6	26.1	28.5	.50
83	245	70	.78	28.2	33.6	15.5	18.1	.84
Generation time 350 minutes								
84	90	235	.28	27.6	61.3	26.3	35.0	.45
Generation time 365–375 minutes								
85	90	250	.26	26.2	58.5	27.3	31.2	.45
86	180	160	.53	31.0	71.4	35.2	36.2	.43§
87	300	45	.87	35.3	40.5	19.4	21.1	.87

TABLE II—*Continued*

Clone No.	Age C_1 (min.)	Time to next div. (min.)	Age C_1 / Interphase*	Amount of DNA (in arbitrary units)				C_1 DNA / C_2 DNA
				C_1	C_2	C_{2a}	C_{2b}	
Generation time 385 minutes								
88	35	325	.10	16.6	38.3	17.6	20.7	.43
Generation time 405 minutes								
89	35	345	.09	18.2	31.8	15.8	16.0	.57
Generation time 480 minutes								
90	300	155	.66	54.4	43.3	20.3	23.0	1.25‡

The deviations to be expected from the ideal C_1/C_2 ratios of 0.5 and 1.0, before and after synthesis, have been calculated from the DNA values of C_{2a} and C_{2b} nuclei, on the assumption that the parent cells (C) may have divided with the same degree of equality as did their daughters (C_2) (Table III). For these calculations, four ratios were used:

(1) low daughter/high daughter × 2 (the situation to be found if the interphase cell contained the lower amount of DNA and had not started synthesis);
(2) high daughter/low daughter × 2 (if the interphase cell contained the higher amount of DNA and had not started synthesis);
(3) low daughter/high daughter (if the interphase cell contained the lower amount of DNA and had completed synthesis); and
(4) high daughter/low daughter (if the interphase cell contained the higher amount of DNA and had completed synthesis).

Obviously any C_1/C_2 ratios of 0.42 or less, or 1.18 or more, must have resulted from divisions approximately as unequal as the 13 most unequal cases listed in Table III. Table II shows three such low and three such high ratios (marked by symbol ‡). In three of these clones (Nos. 3, 65, 66) the C_2 DNA was considerably above or below the mean (51.4 units) for this group of cells. Partial synthesis, obviously, would tend to obscure other such unequal pairs, and asynchronous growth probably would also be reflected by unusual C_1/C_2 ratios. In 7 cases (marked in Table II with symbol §), however, inequality of DNA content is suggested by exceptionally high or low C_2 values, combined with C_1/C_2 ratios unusual for the age of C_1. A total of 13 presumably unequal pairs among the 90 clones are thus designated—which, remarkably, is also the number of the most unequal of the 83 C_{2a}–C_{2b} cells listed in Table III.

From the remainder of Table III it appears that for the bulk of clones, C_1/C_2 ratios of 0.59 or less indicate that C_1 had synthesized little or no DNA, whereas ratios of 0.85 or more indicate that when C_1 was fixed it probably had completed

duplication of its DNA. Based on these figures, the data from Table II have been summarized in Table IV according to (A) the age of C_1, (B) the interphase time elapsing from the fixation of C_1 to the start of the C_2 division, and (C) the relative interphase age of C_1. In the more detailed examination of the clones, below, the numbers refer to Table II.

Consideration of the early part of interphase shows that, of 11 clones in which the C_1 cells were fixed within the first 30–35 minutes, 7 have C_1/C_2 ratios of less than 0.59, indicating that probably no synthesis had occurred. Ratios for the other four clones (Nos. 10, 23, 24 and 56) are all in the low 0.60's. At the other end of interphase, of 40 clones in which C_1 was fixed 80 minutes or less before the next division began, 37 have C_1/C_2 ratios of 0.85 or more, indicating that in these cases synthesis probably had been completed. Two of the three exceptional clones (Nos. 76 and 83) had C_1/C_2 ratios which were only slightly low (0.81 and 0.84). The other clone, No. 63, had a ratio of 0.76; its C_2 value, however, was not unusually high, which suggests the possibility that asynchronous growth of the sister cells had occurred.

Despite the wide range of interphase times among individual cells, it thus appears that DNA synthesis generally does not begin for a period of perhaps a half hour after the previous division, and is completed a considerable length of time—about 80 minutes—before the next division begins. The process of synthesis, then, would occupy a fairly short period of time in cells with short generation times, and a relatively longer period in cells with long generation times. Presumably C_1 cells fixed during this interim would be in the process of synthesizing DNA. Among 39 such clones, 18 do indeed have C_1/C_2 ratios which indicate that partial synthesis had occurred. Nine others have ratios above 0.85, suggesting that synthesis might have been completed when C_1 was fixed. The other 12 have ratios under 0.59, which appear to indicate that no synthesis had occurred. Six of these clones (Nos. 31, 32, 66, 77, 80, and 86), however, had such high C_2 values as to suggest that

TABLE III

Expected C_1/C_2 ratios of DNA at the beginning (before synthesis) and end (after synthesis) of the interphase period, based upon the distribution of the DNA of C_2 between its daughter cells (C_{2a} and C_{2b})

No. of pairs*	No DNA synthesized		All DNA synthesized	
	$\frac{C_{2a}\dagger}{C_{2b} \times 2}$	$\frac{C_{2b}}{C_{2a} \times 2}$	$\frac{C_{2a}}{C_{2b}}$	$\frac{C_{2b}}{C_{2a}}$
5	0.375–.399	0.667–.626	0.750–.798	1.333–1.252
8	.400–.424	.625–.589	.800–.848	1.250–1.178
21	.425–.499	.588–.556	.850–.898	1.176–1.112
20	.450–.474	.555–.527	.900–.948	1.110–1.054
29	.475–.499	.526–.501	.950–.998	1.052–1.002
83 Total				

* Of the 90 clones in Table II, 7 C_2 cells are represented by a single daughter; hence, only 83 pairs of cells are included in this table.

† In Table II, C_{2a} has been listed as the daughter with the lower value of DNA, and C_{2b} as the daughter with the higher value.

TABLE IV

Ratios of DNA in the 90 clones of Table II arranged in different time sequences

	$\frac{C_1\ DNA}{C_2\ DNA}$ ≤ 0.59	$\frac{C_1\ DNA}{C_2\ DNA}$ 0.60–.84	$\frac{C_1\ DNA}{C_2\ DNA}$ ≥ 0.85
	Number of clones		
(A) Age of C_1 when fixed (min.)			
30– 35	7	4	—
40– 65	3	7	1
70– 95	3	5	9
100–135	4	3	7
140–165	1	4	17
170–195	1	—	8
200–225	—	1	2
≥ 230	—	1	2
(B) Time to C_2 div. (after C_1 fixed) (min.)			
≥ 235	4	—	—
205–230	—	—	—
175–200	2	4	—
135–170	6	3	1
105–140	4	7	2
85–110	3	8	6
55– 80	—	3	13
25– 50	—	—	18
≤ 20	—	—	6
(C) $\frac{\text{Age of } C_1}{C_2 \text{ interphase}}$			
0.0–.09	1	—	—
.1–.19	5	2	—
.2–.29	5	6	—
.3–.39	1	4	2
.4–.49	4	3	1
.5–.59	3	4	5
.6–.69	—	3	10
.7–.79	—	3	10
.8–.89	—	—	12
.9–.99	—	—	6

very unequal division of DNA had occurred between the sister cells, thus masking the start of synthesis. In three other cases (Nos. 12, 15, 48), C_1 was fixed so early in interphase (65 minutes or less) that the low ratios probably have little significance. The last three clones (Nos. 82, 84, 85) are unexplained exceptions; possibly the apparent lack of synthesis in C_1 is related to the unusually long generation times.

The data obtained in the course of this study suggest that cells duplicate each unit of DNA during their growth cycles. The presence of 25 clones in the "partial synthesis" group (C_1/C_2 0.60 to 0.84) indicates that the synthesis is not instan-

taneous. In the "completed synthesis" group there are 43 clones with C_1/C_2 ratios of 0.85 to 1.17. (To be sure, some of the low ratios might represent C_1 cells still in the process of synthesis.) Of these 43 clones, 21 have ratios of 0.99 or less (indicating that C_1 contained the lower amount of DNA), 4 have ratios of 1.00, and 18 have ratios above 1.00 (indicating that C_1 contained the higher amount of DNA); selection of the cell with the lower or higher amount of DNA for C_1 was thus perfectly random. In 16 of these clones (37%) the sisters contained nearly equal amounts of DNA (C_1/C_2 ratios of 0.95–1.04); in 12 (28%) the ratios were less equal (0.90–0.94, and 1.05–1.10); and in 15 (35%) the ratios were even less equal (0.85–0.89, and 1.11–1.17). These figures take on special significance when compared with the 70 C_{2a}–C_{2b} cells falling in the same ratio groups (Table III, lines 3, 4, 5)—29 (41%) are in the first group, 20 (29%) in the second, and 21 (30%) in the third. Such close correspondence between these two sets of cells—sisters just at the end of the division process, and sisters of the ensuing life cycle—offers good evidence that, following division, the DNA content of a cell is precisely duplicated.

This investigation of DNA synthesis in *Tetrahymena* indicates, further, that a considerable time elapses from the completion of synthesis to the end of the interphase period. It is intriguing to consider the implications of this time period. Cytological examination shows no obvious, complicated mitotic apparatus being formed, although the chromatin does undergo a fairly regular condensation. Cellular reorganization, however, occurs before the mother cell divides into two daughters. The most striking change which takes place before the start of division is the formation of a new mouth (Furgason, 1940), allotted to the posterior daughter of the dividing pair. By studying growing cells under a phase microscope, it is hoped to determine the amount of time necessary for this process.

Discussion

The synthesis of DNA by *Tetrahymena pyriformis* H clearly occurs during the interphase period. Other workers, in some cases using very different methods of analysis, have come to similar conclusions for cells with mitotically dividing nuclei—the micronuclei of a ciliate (*Chilodonella uncinatus*—Seshachar, 1950), vertebrate cells (Swift, 1950), chick fibroblasts in tissue culture (Walker and Yates, 1952), sea urchin embryos (McMaster, 1955), *Vicia faba* root tips (Howard and Pelc, 1951; Deeley *et al.*, 1957), onion root tips (Patau and Swift, 1953).

After the end of a division (the beginning of the new interphase) a period of time elapses before DNA synthesis begins. This pause was particularly obvious in some of the individual clones with long generation times, when several hours might elapse before synthesis could be detected. Synthesis appears to be completed more than an hour before the cells begin to divide, a considerable period when compared to their average generation time of around four hours.

The exact duplication of DNA during interphase is suggested strongly (1) in mass cultures, by the amounts of DNA in the macronuclei of dividing daughter cells during log phase, as compared with the amounts in older, non-dividing cells, and (2) in individual clones, by the similar DNA ratios in pairs of dividing daughters, as compared with those in sister pairs after synthesis of DNA had occurred.

In general, the amounts of DNA allotted to sister nuclei are remarkably close. Frequently, however, division is quite unequal; furthermore, very often a piece of chromatin is left behind in the cytoplasm. Such irregularities might be expected to result in genic imbalance, and death; instead, they seem to result in the wide range of DNA values found among non-clonal cells. Very occasional deaths have been noted among isolated cells (2 among a set of 101 cells, for example), which might be attributable either to cellular abnormality, or to some external factor. Among cells fixed during growth, occasional diffuse-looking nuclei have been found which were unsuitable for photometric analysis—possibly they represented dying cells, or possibly they resulted from faulty fixation. That unequal division of the macronucleus does not usually have an adverse effect is shown by the fact that the DNA ratios between apparently healthy sister cells, after synthesis of new DNA, are as variable as those between dividing daughters. It seems reasonable, however, that some method for a fairly orderly distribution of chromatin must be present in this micro-organism, with no micronucleus but only an amitotically dividing macronucleus, which has successfully propagated itself for almost 30 years in the laboratory. At division, the appearance of the *Tetrahymena* macronucleus does suggest some sort of organization.

Unfortunately, the basic structure of ciliate macronuclei is difficult to interpret. In *Paramecium aurelia,* however, Sonneborn (1947) obtained genetic evidence (the regeneration of parts of degenerating macronuclei) for genome segregation, from which he concluded that the macronucleus must contain about 40 diploid "subnuclei," distributed at amitotic division in intact units. (It will be recalled that Moses, 1950, estimated the macronucleus of *P. caudatum* to contain about 40 times as much nucleoprotein as the micronucleus.) Kimball (1953) could find no cytological evidence for subunits in the macronucleus of *P. aurelia,* however, but only for a high degree of polyploidy.

Grell, too, has been unable to find cytological evidence for subnuclei in the macronuclei of suctoreans (for example, Grell, 1953b). The budding by which these organisms reproduce vegetatively, however, suggests that genome segregation must occur. Particularly in the free-living stage of *Tachyblaston ephelotensis,* Grell (1950) noted the similarity in size and structure of the parts budded off the parent macronucleus, with its linear arrangement of chromatin (he reported that there appeared to be 8 chromatin elements in each bud). In another type of protozoan—a radiolarian, *Aulacantha scolymantha*—Grell (1953c) has found clear cytological evidence for a method by which genome segregation could occur. In the highly polyploid nucleus of this organism, the chromosomes appear to be linearly arranged in complete, individual sets, forming numerous chains of "Sammelchromosomen." Random separation of these intact genomes necessarily would result in perfectly balanced daughter nuclei. It is possible that the occurrence of "Sammel" chromosomes might also explain the efficiency of amitosis in ciliates, although the division figure of the *Aulacantha* nucleus, which also has no spindle, appears to be quite different from those of ciliate macronuclei.

Purely in the realm of speculation, it has occurred to the present author that, rather than "Sammel" chromosomes, the Feulgen-positive granular strands which appear to extend the length of the dividing macronucleus in *Tetrahymena* might each represent a row of identical chromosomes, held together by forces of attraction. If this were the case, the daughter nuclei would be assured of a fairly well balanced

assortment of chromosomes, no matter how unequal the division (assuming that all the strands separated in approximately the same region). The chromatin fragments left behind at division would probably cause no serious imbalance, and might, indeed, be a way of correcting a nucleus somewhat imbalanced by the previous division. If a similar chromosome arrangement also occurred in the degenerating macronuclear skein of *Paramecium,* the parts breaking off would very likely contain complements of chromosomes, as has been suggested by the regeneration experiments. A condition such as this could also explain the efficiency of the budding of suctoreans.

Another characteristic of *T. pyriformis* H which has been demonstrated in the present experiments is the variability in generation times among different clones, as compared with the usual close similarity between sister cells. The length of generation time seems to have no relation to the amount of DNA.

Once synthesis of DNA has been completed, the photometric measurements of mass culture cells indicate that under some conditions division does not necessarily follow immediately. In general, cells from the one-week- and two-week-old culture contained approximately twice as much DNA as individual dividing daughters from log phase. Prescott's recent studies (1957) of generation time and lag phase in strain HS indicate that cells from stationary phase may divide soon after inoculation into fresh medium, and may then undergo a lag phase before logarithmic growth begins. Such preliminary division seems a reasonable consequence if the inoculated cells contained a doubled amount of DNA.

By alternating temperature changes, Scherbaum and Zeuthen (1954) caused logarithmically growing cells (strain GL) essentially to stop dividing until the final return to optimal temperature. The following synchronous (85%) division occurred 90 ± 10 minutes later, a time period remarkably similar to that found in the present experiments (about 80 minutes) to occur between the end of synthesis and the end of the interphase period. They report that the following two somewhat less synchronous divisions of the treated cells occurred about 1.7 hours (100 minutes) apart. Correlated with these interesting data is the fact that they found (Zeuthen and Scherbaum, 1954) by Hoff-Jørgensen microbiological assay that cells at the end of treatment contained about four times as much DNA as normally growing cells. By Schmitt-Thannhauser analysis, Ducoff (1956) has confirmed the unusual amount of DNA synthesis by temperature-treated cells. In view of the degree of DNA synthesis and the time elapsing before the first synchronous division, the question arises, as in the present experiments, about the length of time required for cellular changes (such as the formation of a new mouth) which must take place before division can begin.

The author wishes to express her appreciation to Professor A. W. Pollister, in whose laboratories this work was carried out, for his stimulating advice and guidance, and to Professor F. J. Ryan for the provision of additional laboratory facilities.

Summary

1. In a mass culture of *Tetrahymena pyriformis* H which has stopped growing, the macronuclei contain approximately twice as much DNA as do newly divided macronuclei in a logarithmically growing culture.

2. Non-clonal cells show considerable variability as to DNA content and generation time.

3. Cells in small clones show close similarity as to DNA content and generation time.

4. Duplication of DNA occurs during an intermediate part of interphase, starting some time after the end of the previous cell division, and reaching completion a considerable period of time before the next division begins.

LITERATURE CITED

ALFERT, M., AND I. I. GESCHWIND, 1953. A selective staining method for the basic proteins of cell nuclei. *Proc. Nat. Acad. Sci.*, **39**: 991–999.

ALFERT, M., AND N. O. GOLDSTEIN, 1955. Cytochemical properties of nucleoproteins in *Tetrahymena pyriformis;* a difference in protein composition between macro and micronuclei. *J. Exp. Zool.*, **130**: 403–421.

CHEN, T. T., 1940. Conjugation in *Paramecium bursaria* between animals with very different chromosome numbers and between animals with and without micronuclei. *Proc. Nat. Acad. Sci.*, **26**: 243–246.

CHEN, T. T., 1944. Staining nuclei and chromosomes in protozoa. *Stain Tech.*, **19**: 83–90.

CORLISS, J. O., 1953. Comparative studies on holotrichous ciliates in the *Colpidium-Glaucoma-Leucophrys-Tetrahymena* group. II. Morphology, life cycles and systematic status of strains in pure culture. *Parasitology*, **43**: 49–87.

DEELEY, E. M., H. G. DAVIES AND J. CHAYEN, 1957. The DNA content of cells in the root of *Vicia faba*. *Exp. Cell Res.*, **12**: 582–591.

DI STEFANO, H. S., 1948. A cytochemical study of the Feulgen nucleal reaction. *Chromosoma*, **3**: 282–301.

DUCOFF, H. S., 1956. Radiation-induced fission block in synchronous cultures of *Tetrahymena pyriformis* W. *Exp. Cell Res.*, **11**: 218–220.

ELLIOTT, A. M., AND R. E. HAYES, 1953. Mating types in *Tetrahymena*. *Biol. Bull.*, **105**: 269–284.

FURGASON, W., 1940. Significant cytostomal pattern of "*Glaucoma-Colpidium* group" and a proposed new genus and species, *Tetrahymena geleii*. *Arch. f. Protist.*, **94**: 224–266.

GRELL, K. G., 1950. Der Generationswechsel des parasitischen Suktors *Tachyblaston ephelotensis* Martin. *Zeitschr. f. Parasit.*, **14**: 499–534.

GRELL, K. G., 1953a. Die Konjugation von *Ephelota gemmipara* R. Hertwig. *Arch. f. Protist.*, **98**: 287–326.

GRELL, K. G., 1953b. Die Struktur des Makronucleus von *Tokophrya*. *Arch. f. Protist.*, **98**: 466–468.

GRELL, K. G., 1953c. Die Chromosomen von *Aulacantha scolymantha* Haeckel. *Arch. f. Protist.*, **99**: 1–54.

HETHERINGTON, A., 1933. The culture of some holotrichous ciliates. *Arch. f. Protist.*, **80**: 255–280.

HOWARD, A., AND S. R. PELC, 1951. Nuclear incorporation of P^{32} as demonstrated by autoradiographs. *Exp. Cell Res.*, **2**: 178–187.

KIDDER, G. W., V. C. DEWEY AND M. R. HEINRICH, 1954. The effect of non-ionic detergents on the growth of *Tetrahymena*. *Exp. Cell Res.*, **7**: 256–264.

KIMBALL, R. F., 1953. The structure of the macronucleus of *Paramecium aurelia*. *Proc. Nat. Acad. Sci.*, **39**: 345–347.

MCMASTER, R. D., 1955. Desoxyribose nucleic acid in cleavage and larval stages of the sea urchin. *J. Exp. Zool.*, **130**: 1–27.

MOSES, M. J., 1950. Nucleic acids and proteins in the nuclei of *Paramecium*. *J. Morph.*, **87**: 493–536.

NANNEY, D. L., 1953a. Mating type determination in *Paramecium aurelia*, a model of nucleocytoplasmic interaction. *Proc. Nat. Acad. Sci.*, **39**: 113–119.

NANNEY, D. L., 1953b. Nucleocytoplasmic interaction during conjugation in *Tetrahymena*. *Biol. Bull.*, **105**: 133–148.

NANNEY, D. L., 1956. Caryonidal inheritance and nuclear differentiation. *Amer. Nat.*, **90**: 291–307.

NANNEY, D. L., 1957. The role of cytoplasm in heredity. *In:* A Symposium on the Chemical Basis of Heredity, W. D. McElroy and B. Glass, eds., The Johns Hopkins Press, Baltimore, 134–164.

NANNEY, D. L., AND P. A. CAUGHEY, 1953. Mating type determination in *Tetrahymena pyriformis*. *Proc. Nat. Acad. Sci.*, **39**: 1057–1063.

ORNSTEIN, L., 1952. The distributional error in microspectrophotometry. *Lab. Invest.*, **1**: 250–265.

PATAU, K., 1952. Absorption microphotometry of irregular-shaped objects. *Chromosoma*, **5**: 341–362.

PATAU, K., AND H. SWIFT, 1953. The DNA content (Feulgen) of nuclei during mitosis in a root tip of an onion. *Chromosoma*, **6**: 149–169.

POLLISTER, A. W., 1952. Photomultiplier apparatus for microspectrophotometry of cells. *Lab. Invest.*, **1**: 106–114.

PRESCOTT, D. M., 1957. Change in physiological state of cell population as a function of culture growth and age (*Tetrahymena geleii*). *Exp. Cell Res.*, **12**: 126–134.

SCHERBAUM, O., AND E. ZEUTHEN, 1954. Induction of synchronous cell division in mass cultures of *Tetrahymena pyriformis*. *Exp. Cell Res.*, **6**: 222–227.

SESHACHAR, B. R., 1950. Desoxyribonucleic acid content of the ciliate micronucleus. *Nature*, **165**: 848.

SESHACHAR, B. R., AND C. M. S. DASS, 1953. Macronuclear regeneration in *Epistylis articulata*. *Quart. J. Micr. Sci.*, **94**: 185–192.

SONNEBORN, T. M., 1939. *Paramecium aurelia:* mating types and groups; lethal interactions; determination and inheritance. *Amer. Nat.*, **73**: 390–413.

SONNEBORN, T. M., 1947. Recent advances in the genetics of *Paramecium* and *Euplotes*. *Adv. in Genetics*, **1**: 263–358.

SONNEBORN, T. M., 1951. Some current problems of genetics in the light of investigations on *Chlamydomonas* and *Paramecium*. *Cold Spring Harb. Symp. Quant. Biol.*, **16**: 483–503.

SONNEBORN, T. M., 1953. Patterns of nucleocytoplasmic integration in *Paramecium*. *Proc. 9 Intern. Congr. Genetics, Caryologia*, **6** (Suppl. I): 307–325.

SONNEBORN, T. M., 1954. Is gene K active in the micronucleus of *Paramecium?* *Microb. Genet. Bull.*, **11**: 25–26.

SWIFT, H., 1950. The desoxyribose nucleic acid content of animal nuclei. *Physiol. Zoöl.*, **23**: 169–198.

SWIFT, H., 1953. Quantitative aspects of nuclear nucleoproteins. *Int. Rev. Cyt.*, **2**: 1–76.

WALKER, P. M. B., AND H. B. YATES, 1952. Nuclear components of dividing cells. *Proc. Roy. Soc. London, Ser. B*, **140**: 274–299.

WICHTERMAN, R., 1953. The Biology of Paramecium. The Blakiston Co., New York.

ZEUTHEN, E., AND O. SCHERBAUM, 1954. Synchronous divisions in mass cultures of the ciliate protozoan *Tetrahymena pyriformis* as induced by temperature changes. *In:* Recent Developments in Cell Physiology, J. A. Kitching, ed.; Proceedings of the 7th Symposium of the Colston Research Society, London, 141–157.