SYNTHESIS OF DEOXYRIBONUCLEIC ACID BY MICRO- AND MACRONUCLEI OF *TETRAHYMENA PYRIFORMIS*

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ABSTRACT

Evidence as to the times of DNA synthesis in micronucleate *Tetrahymena pyriformis* (mating type II, variety 1) has been obtained by briefly exposing individuals of different ages to tritiated thymidine, returning them to non-radioactive medium, fixing at division, and preparing autoradiographs. A variable length of interphase, ranging from a few minutes to about 2 hours, has been found to precede the initiation of macronuclear DNA synthesis. Once begun, however, the period of synthesis appears to be similar in all cells, regardless of generation time, and has been estimated at 1 to 1½ hours. Under the conditions of these experiments, the time elapsing between the end of synthesis and subsequent division into daughter cells ranges from approximately 1½ to 2½ hours in generation times long enough to allow such variability. Division of the micronucleus occurs shortly before the cell begins to divide; its DNA synthesis starts immediately and continues after cell division for a total period estimated at about an hour.

Most cells contain a single nucleus; not so the typical ciliate, which has at least two—a small diploid micronucleus, and a massive polyploid macronucleus. Most nuclei complete mitosis at about the time the cell divides; the ciliate nuclei are generally out of phase, with the micronucleus dividing first, mitotically, and the macronucleus pulling apart, amitotically, when the cell constricts.

The doubling of deoxyribonucleic acid (DNA) usually is a necessary prelude to nuclear division and, in a variety of cell types studied by microspectrophotometry and autoradiography, has been found to be a discontinuous process which occurs during interphase (for example, 3, 6–12, 14–18, 22, 23, 25). When generation times have been measured, it has proved possible to establish rather precisely the parts of the life cycle allotted to presynthesis, synthesis, and postsynthesis.

In the present experiments, DNA synthesis in micronucleate *Tetrahymena pyriformis*, of variable generation time, has been studied by means of tritiated thymidine autoradiography. It has been possible to determine that the dimorphic nuclei of this ciliate perform their syntheses at markedly different times, and to relate these periods to the diverse life spans of the cells. A preliminary report of some of this work has been published previously (10).

MATERIALS AND METHODS

Mating type II, variety 1, of *Tetrahymena pyriformis*, kindly supplied by Dr. Sally Lyman Allen of the University of Michigan, has been used throughout these experiments. Cultures were grown in 1 per cent proteose peptone broth (Difco Laboratories Inc., Detroit) at 25°C as previously described (9). Experimental cells were removed from day-old cultures to drops of medium in small watch glasses (United States Bureau of Plant Industry model, Arthur H. Thomas Co., Philadelphia), which were placed within inverted Petri plates containing a thin layer of water. Both dividing cells and interphase

cells, which showed no visible signs of division, were isolated. In many experiments, a number of cells in a similar stage of division, which separated within 10 minutes or less of each other, were placed in a single drop of medium, for later simultaneous exposure to tritiated thymidine. Generation times of cells isolated during division tended to be longer (632 cells: range 160 to 505 minutes, mean 276, median 270) than those of cells isolated during interphase (333 cells: range 150 to 325 minutes, mean 217, median 212). These observations are in accord with Prescott's report that *Tetrahymena* removed from log phase cultures more than about 10 hours old show a lag while they accommodate themselves to fresh medium (13). Such a lag in the dividing isolates of the present experiments would have occurred before their second division, and in the interphase isolates usually before their first division (for the latter cells, the interval elapsing between isolation and first division often was longer than the subsequent generation time).

At different ages after division, cells were placed in medium supplemented with 10 μc/ml tritiated thymidine (TDRH^3, specific activity 360 mc/mmole, lot 1191, Schwarz BioResearch, Inc., Mount Vernon New York); some were fixed after 5 to 10 minutes; others remained in radioactive medium until the start of the next division, when they were fixed; but most were exposed for 5 to 10 minutes, and were then rinsed twice and returned to non-radioactive medium until their division, when they were fixed. For the last two groups, approximate generation times could thus be estimated; in addition, micro- and macronuclei, typically widely separated in a dividing cell, usually could be clearly distinguished (a considerable problem, for example, in an autoradiograph of a cell fixed during interphase, with the micronucleus lying close to a highly radioactive macronucleus).

Comparison of sister cell generation times (which, as in amicronucleate strain H (9), tend to be rather similar) indicated that brief treatment with TDRH^3, followed by rinsing and replacement in non-radioactive medium, increased generation time no more than did simple transfer to a fresh drop of medium, an average of less than 10 minutes (estimate for each group based on 20 pairs, with one sister of each pair serving as a control, and one as the experimental cell).

At fixation, individual cells were rinsed twice in water, placed on a "subbed" slide (Kodak Autoradiographic Stripping Film direction sheet PL1157 R1085), and allowed to dry. After all the desired cells had been positioned on the slide, they were postfixed in 3:1 Carnoy solution. Such air-dried cells usually flatten to a rather circular shape, about 45 to 60 μ in diameter, with the somewhat misshapen macronucleus occupying about a tenth of the area. Cells similarly air-dried on Saran Wrap (Dow Chemical Co., Midland, Michigan) and then postfixed, stained, paraffin embedded, and sectioned, usually measured in total thickness about 2.25 μ (maximum 3 μ), their macronuclei averaging 1.75 μ (maximum 2.25 μ), and cytoplasm overlying the macronuclei averaging 0.25 μ (maximum 0.5 μ).

Prior to the application of autoradiographic stripping film (AR.10, Kodak Ltd, London), most nuclei were stained by the Feulgen method (21), but comparable autoradiographs were obtained for both stained and unstained cells. Slides were developed (22) after 1 to 2 weeks exposure.

RESULTS

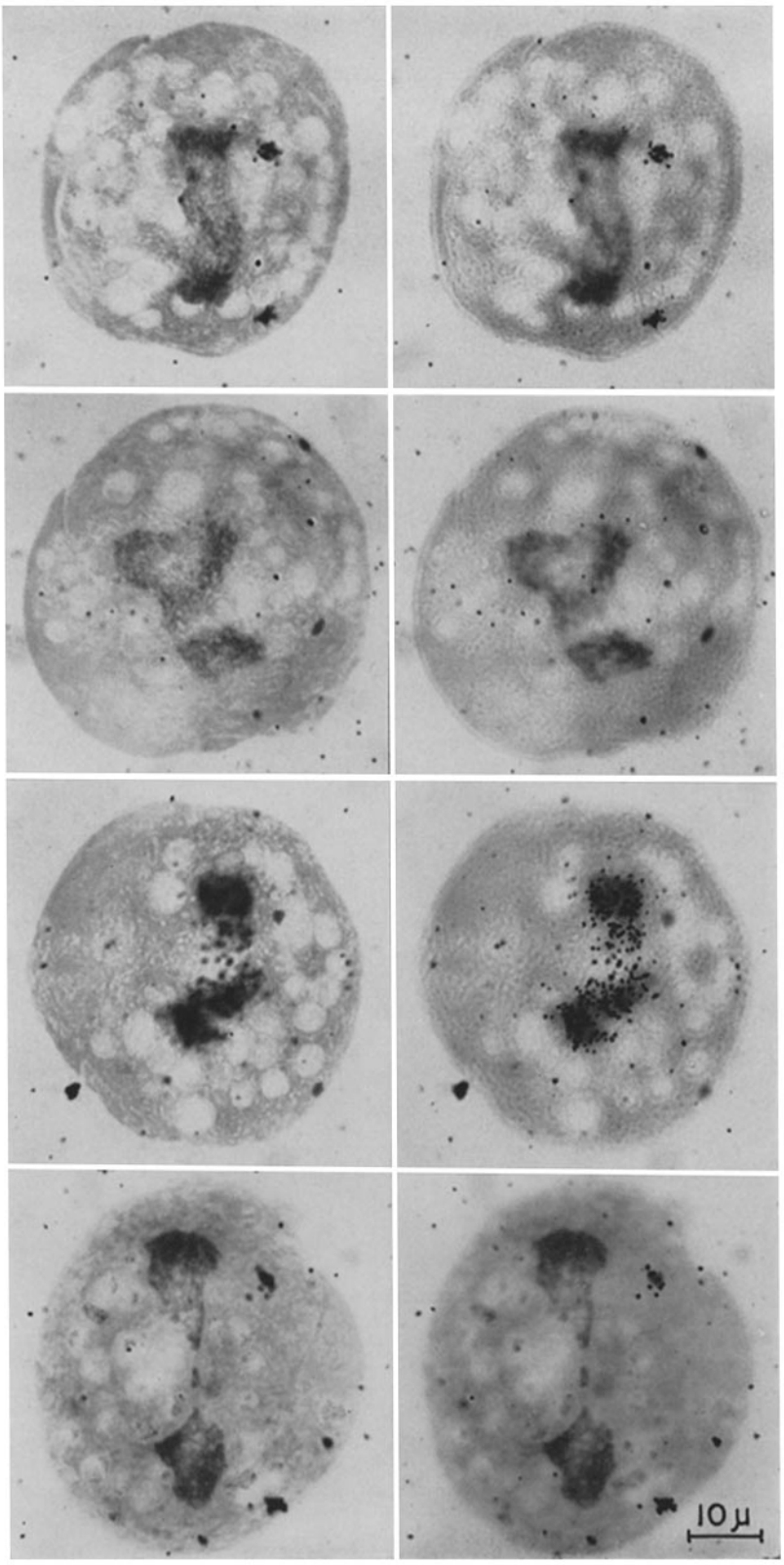
In Fig. 1 are shown autoradiographs of *Tetrahymena* treated briefly at various ages with TDRH^3, and, except for the last example (D), returned to non-radioactive medium until division. The two micronuclei are toward the right in each case. Cell A was 5 minutes old when exposed to TDRH^3, and shows micronuclear labeling only; cell B was 30 minutes old, and contains no nuclear activity; cell C was 60 minutes old, and has incorporated TDRH^3 into the macronucleus only; cell D was in a very early stage of division, and, like cell A, shows activity restricted to the micronuclei. Active macronuclei of *Tetrahymena*, as represented by cell C, have not revealed regional incorporation of thymidine such as that which occurs in the "reorganization bands" of the ciliate *Euplotes* (2).

From these autoradiographs it can be seen that the macronucleus synthesizes DNA during cellular interphase, whereas the micronucleus, whose division precedes that of the cell, begins to synthesize DNA before the cell divides, and continues for a short time afterward.

FIGURE 1

Autoradiographs of *Tetrahymena* exposed to tritiated thymidine at different ages, and (except for D) replaced in non-radioactive medium until division. A was treated at 5 minutes, B at 30 minutes, C at 60 minutes, and D at the start of division. The two micronuclei are toward the right in each cell. Focal level of photographs at left on cells, and at right on emulsion.

Feulgen nuclear stain, eosin counterstain.


A
B
C
D
10 μ

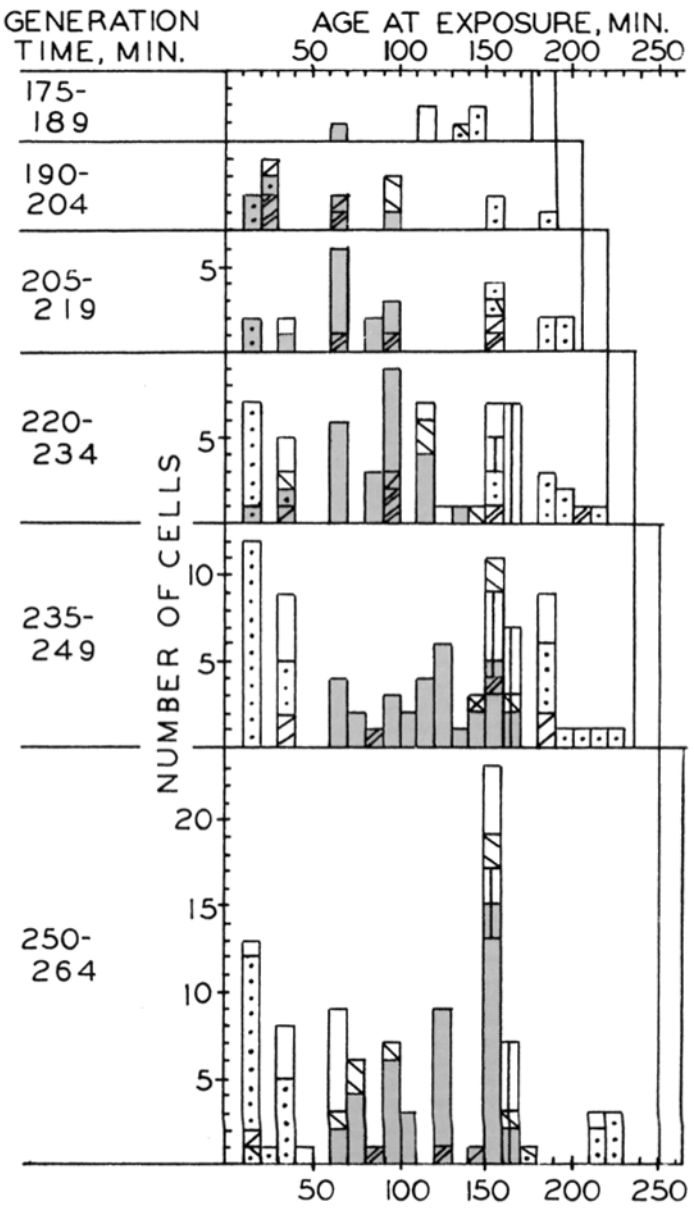


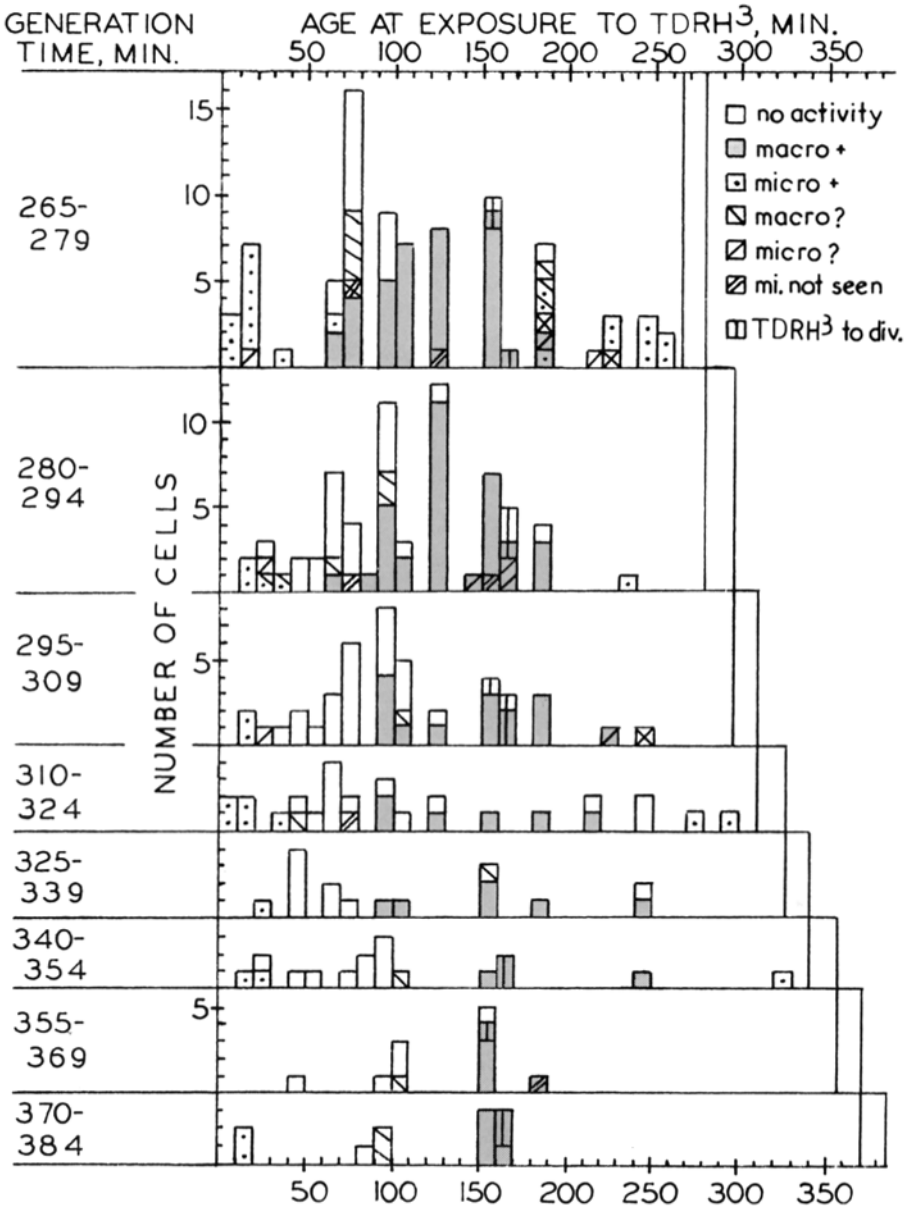
FIGURE 2

A representation of the data obtained from autoradiographs of *Tetrahymena* exposed to tritiated thymidine ($TDRH^3$) at different ages. Each histogram includes cells having generation times within a certain 15 minute range.

no activity: the isotope was not utilized by one or both types of nuclei.

macro + and *micro* +: the designated nucleus incorporated $TDRH^3$ and was therefore synthesizing DNA.

The periods of synthesis for cells of different generation times are represented in some detail by the histograms of Fig. 2. Unless symbolized to the contrary (see below), the cells included in this figure were exposed briefly to $TDRH^3$ at the indicated ages, and then returned to non-radioactive medium until division. Generation times, from 175 to 384 minutes, are grouped into 15 minute



macro ? and *micro ?:* activity was possibly present in the designated nucleus, but was very low and close to background.

mi. not seen: the micronucleus was not seen.

TDRH³ to div.: these cells remained in radioactive medium until they divided.

Except for the last group, all the cells in this figure were treated briefly with TDRH³ and were then returned to non-radioactive medium until fission began. Since the cells were fixed early in division, their separation times were estimated.

periods (9 cells of shorter and longer life spans have been omitted), with the time range indicated at the end of each group. For the individual cells, symbols indicate whether or not activity was incorporated into one or both types of nuclei. In some cells, however, as shown by the figure, "activity" was so close to background that it was considered to be questionable (complicating the

interpretation of possible low nuclear activity is the occurrence of extranuclear activity, not extracted with deoxyribonuclease (Worthington Biochemical Corp., Freehold, New Jersey), which is often observed in cells exposed to radioactive medium late in the life cycle). In other cells, the micronuclei were not seen (sometimes, at least, because of proximity to an active macronucleus). Finally, symbolized in Fig. 2, in addition to cells treated briefly with $TDRH^3$, are some which were left in radioactive medium from 150 or 160 minutes of age until division, to provide information as to whether or not macronuclear synthesis had been completed by the time of initial exposure (their micronuclei are not represented, being invariably active by the time of fixation).

While the completion of DNA synthesis presumably would be indicated fairly accurately in Fig. 2 by cells which shunned the isotope, its beginning might well be obscured if cells, treated prior to the onset of synthesis, retained $TDRH^3$ in food vacuoles or in a precursor pool for later use. To test this possibility, at different times following division, groups of cells of nearly identical ages (having been isolated together while dividing) were briefly exposed to $TDRH^3$; some were immediately fixed, and others were returned to nonactive medium until division. At most of the ages tested, comparable autoradiographs were produced by the two types of cells, but some variation occurred at 60 minutes for macronuclei and at 180 minutes for micronuclei, as shown in Table I (adjacent treatment times are included for comparison). At these two periods, the greater number of labeled cells among those that went on to division, as compared with cells fixed after treatment, indicates that $TDRH^3$ may indeed be temporarily stored for later use. The close agreement between cells treated 30 minutes prior to the two critical times, however, suggests that such storage is of limited duration—for the macronucleus probably less than 30 minutes, since cells of both types treated at 90 minutes produced similar autoradiographs. (Data were not obtained at 210 minutes for the micronucleus.) In estimating from Fig. 2 the times at which DNA synthesis begins, therefore, allowance must be made for post-treatment incorporation of $TDRH^3$.

Whether post-treatment incorporation might also account for some organisms which utilized $TDRH^3$ in both nuclei (Fig. 2: short life cycles, treated while very young) was investigated by placing cells 15 or 20 minutes old in radioactive medium and then fixing them. Of 24 so treated, 2 clearly contained activity in both nuclei, 1 contained no activity in either nucleus, and the others contained micronuclear activity only. From these results it appears that both nuclei of a cell can simultaneously synthesize DNA (or at least can do so within a 5 minute exposure period, plus a maximum of 4 minutes, in this experiment, for washing and drying).

TABLE I

Nuclear Status of Tetrahymena Exposed Briefly to Tritiated Thymidine at Different Ages, and either Fixed Immediately or Fixed at the Following Division

Age when first exposed (min.)	Fixed	Total no. of cells	No. of cells with Macro +	Macro −
30	Immediately*	21	1	20
	At division	16	1	15
60	Immediately	18	0	18
	At division	18	8	10
90	Immediately	19	18	1
	At division	15	14	1
			Micro +	Micro −
150	Immediately	17	0	17
	At division	18	1	17
180	Immediately	8	1‡	7
	At division	15	6	9

* After 5 to 6 minutes exposure and up to 5 minutes for washing and drying.

‡ The micronucleus in this cell had divided.

Micronuclear Events Related to the Life Cycle

The demonstration of post-treatment incorporation of $TDRH^3$ signifies that DNA synthesis begins later than is indicated by the cells of Fig. 2. Synthesis by the micronucleus would be expected to start only after it had divided and returned to interphase. In order to find out how long before fission the micronucleus does divide, sister cells were studied: at an age of about 150 minutes, one sister of each pair was fixed (in some cases following brief exposure to $TDRH^3$) and examined for micronuclear status, and the other's subsequent division was timed on the assumption that it would indicate the potential division time of its fixed twin. Among 39 cells, separation occurred 14 to 127 minutes after the first sister had been fixed. In

only 6 cases had the micronucleus divided (sister separations 14, 29, 32, 34, 35 minutes later), but in 21 cells (sister separations 27 to 127 minutes later) the micronucleus had moved away from the macronucleus and assumed a predivision, ellipsoid-spindle shape (4). (In the other 12 cases the micronuclear configuration was not clearly distinguishable.) Of 3 cells which had been exposed to $TDRH^3$ and contained divided micronuclei, 2 (sisters dividing 29 and 35 minutes later) had widely separated, spherical, radioactive micronuclei, and the other (sister dividing 14 minutes later) had a micronucleus still in anaphase, and inactive. It appears, therefore, that micronuclear division may precede separation of the cell by about a half hour, and that DNA synthesis by the micronucleus probably begins as soon as mitosis is completed. Although the visible process of fission lasts, on the average, about 20 minutes, it is somewhat variable. For this reason, a few minutes' difference might be expected in the length of time by which micronuclear division and the start of DNA synthesis precede cell separation. Assuming the period of synthesis to be constant, this difference also would be reflected in the length of time that synthesis continues in daughter cells.

Micronuclear activity in cells of Fig. 2, treated with $TDRH^3$ shortly after division, does indicate such variation, but in general synthesis appears to have been completed at an age of about 30 minutes. Added to the approximately 30 minute interval prior to division, this suggests that the total period of synthesis is about an hour in length. (The micronucleus of one exceptional cell, 277 minutes generation time, incorporated $TDRH^3$ at 60 minutes of age, which might mean that the length of synthesis was greater than usual, that the previous mitosis, in relation to fission, was later than usual, or that it was followed by an unusually long pause before synthesis began.)

For further information as to when the micronucleus moves away from the macronucleus and assumes an ellipsoid shape, samples of three log phase mass cultures were fixed (9 parts absolute alcohol to 1 part glacial acetic acid (9)) and stained, and from each sample 100 cells were classified which showed evidence of dividing micronuclei, from early ellipsoid up through nearly separated daughter cells. Close to 70 per cent (*i.e.*, 69 ± 5 per cent) of the cells contained micronuclei preparing to divide, and 30 per cent contained micronuclei in various stages of division. The latter figure of 30 per cent is conveniently similar to the approximately 30 minute period by which division of the micronucleus appears to precede separation of the cell; the 70 per cent of cells having predivision figures might then indicate that a roughly 70 minute interval of prophase and metaphase occurs before the micronucleus divides.

If these assumptions are justified, the micronucleus would begin preparations to divide about 70 minutes before it actually did so; once divided, its *G*1 (6) would be very short, because it appears to begin DNA synthesis almost immediately; *S* would be about an hour long; and *G*2 would be variable as the cell's generation time is variable, being terminated when the micronucleus again entered prophase.

Macronuclear Events Related to the Life Cycle

In Fig. 2 it can be seen that as generation time lengthens, an increasing postdivision period (*G*1)[1] intervenes before incorporation of $TDRH^3$ is detected in macronuclei. This period can be correlated with the "lag" exhibited particularly by cells which had been isolated at division (predominantly those in Fig. 2 with generation times longer than 234 minutes). Disregarding for the moment post-treatment incorporation of $TDRH^3$, it appears that in the different generation time groups most of the cells synthesizing macronuclear DNA fall within a time range of 130 minutes or less, which suggests a similar length of *S*, regardless of generation time, occurring within a comparable period prior to the ensuing division. At the extremities of the synthetic periods are cells which did and did not incorporate $TDRH^3$. Discounting some cells of questionable activity, synthesis appears to have stopped by 80 or 90 minutes before division (*G*2) in all groups.

Information pertinent to *G*2 can be obtained from cells which remained in radioactive medium from 150 or 160 minutes of age until division, as represented in Fig. 2, and summarized in Table II. In none of the cells dividing less than 80 minutes after introduction into the radioactive medium was macronuclear activity detected; the first to

[1] For convenience in discussion, the relationships of the life cycle and macronuclear synthesis are treated here as three intervals: *G*1, the time between cell separation and the start of macronuclear synthesis; *S*, the period of synthesis; and *G*2, the time elapsing from the end of synthesis to cell separation (symbols of Howard and Pelc (6)). Since the visible process of division takes about 20 minutes, *G*2 actually would be shorter by at least that length of time.

reveal such activity divided 85 to 90 minutes later. From that time, up to about 140 minutes, are distributed cells with and without macronuclear activity. The generation times of these cells were similar to those of other isolated groups, and so there is no reason to suspect that the long sojourn in radioactive medium altered the growth rate to an appreciable extent; therefore, it appears that in the *Tetrahymena* of these experiments the *G*2 interval could vary from about 1½ to nearly 2½ hours.

TABLE II

Macronuclear Status of Tetrahymena Remaining in Radioactive Medium from 150 or 160 Minutes of Age to Division

Time elapsing from initial exposure to cell division (min.)	Macronuclear activity (no. of cells)		
	+	−	?
60–69		7	
70–79		3	
80–89	3	8	1
90–99	2	3	1
100–109	3	2	
110–119	1	1	
120–129	1	2	
130–139	1	1	
140–149	1	1	
. . .			
180–189	2		
. . .			
200–209	3		
. . .			
230–239	1		

Such variability in *G*2 substantiates the possibility that *S* is approximately the same in all cells; within a generation time group those cells which began synthesis early (after a relatively short *G*1) might well have undergone longer *G*2 periods than those which started synthesis later. To facilitate consideration of *S*, in Fig. 3 *a* are compiled the percentages of cells from Fig. 2, having intermediate life spans of 220 to 279 minutes, which were synthesizing macronuclear DNA at different times of treatment, as calculated back from the time of the subsequent division. (Cells of questionable activity were considered to be negative.) *G*1 is not extensively represented in this figure, being variable according to the generation times of the cells. The distribution of *S* is approximately symmetrical, which again might indicate that all cells have a similarly long synthetic period. These data are replotted in Fig. 3 *b*, with the descending frequencies of Fig. 3 *a* represented on an inverted scale, to illustrate the situation which would exist if *S* were similar in all cells and were followed by *G*2 varying from 80 to 120 minutes, as in the cells of this figure. The relative times at which *S* and *G*2 would be initiated, for cells with different *G*2 intervals, can be traced across the horizontal axis

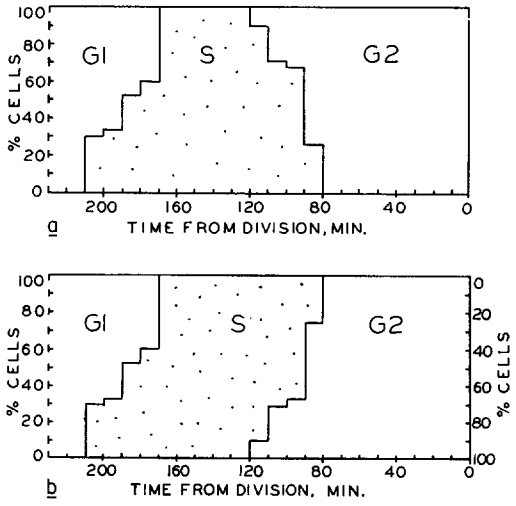


FIGURE 3

a. The distributions of macronuclear *S* and *G*2 (and part of *G*1) for cells from Fig. 2 which had generation times of 220 to 279 minutes. In *S* are represented the percentages of cells which incorporated tritiated thymidine into their macronuclei at periods calculated back from the time of the subsequent division.

b. Same as *a*, but with the descending frequencies at the right side of *a* (percentage of cells in *S* at different times) plotted in reverse, illustrating the likelihood that the period of macronuclear DNA synthesis is similar in all cells (*i.e.*, cells with a short *G*2 began synthesis later than cells with a long *G*2, etc.).

of Fig. 3 *b*. (As indicated in Fig. 2 and Table II, cells with generation times above 280 minutes can have *G*2 periods as long as 140 to 150 minutes. Conversely, with a standard *S* and minimum *G*2, cells with very short generation times necessarily would have less variable *G*2.)

If this interpretation of the data is correct, macronuclear *S* would appear to be about 90 minutes long. Adjustment must be made, however, for post-treatment incorporation of TDRH[3] in cells exposed to radioactive medium shortly

before the beginning of synthesis. Allowing for this, *S* undoubtedly is less than 90 minutes. If it were actually 70 minutes, and the minimum *G*2 were 80 minutes, and *G*1 could be very brief (as in the case of the micronucleus), then the shortest possible generation time for cells grown under these conditions would be 150 minutes; occasionally cells have been observed with generation times as short as this. If 70 minutes does approximate *S* for the macronucleus, then it would appear that, despite their discrepant sizes, both the macronucleus and the micronucleus of *Tetrahymena pyriformis* require similar periods of time in which to perform DNA synthesis.

DISCUSSION

These experiments with *Tetrahymena pyriformis*, mating type II, variety 1, have indicated that for the macronucleus *G*1 may range from a few minutes to 2 hours or more, that *S* (probably similar in all cells) is between 1 and 1½ hours long, and that *G*2 (including division time of about 20 minutes) ranges from 1½ to 2½ hours. In contrast, synthesis by the micronucleus begins with its division just before, and ends after, fission: *G*1 is very short, *S* is about an hour long, *G*2 (combining parts of macronuclear *G*1, *S*, and *G*2) is highly variable, and prometaphase is perhaps 70 minutes long. Although the two nuclei initiate DNA synthesis at very different periods, thus, both accomplish the task in similar spans of time.

In having particularly variable *G*1, similar *S*, and moderately variable *G*2, these *Tetrahymena* macronuclei resemble the nuclei in populations of other cells, studied both *in vivo* (including tumors (11), intestinal epithelium (15), and ear epidermis (17) of mice) and *in vitro* (including various types of tissue cultures (for example, 16, 18); evidence of marked variability in *G*2, and some in *S* (at least partly due to differences in growth medium), however, has been obtained for HeLa cells (12)).

A basic growth pattern for many cells seems to have been revealed. That the macronucleus fits into this pattern is not surprising, since it has long been considered the functional nucleus of ciliates during normal vegetative growth (see 19). Although the micronucleus differs from other nuclei in conducting DNA synthesis at the time of cell division, it has, of course, entered its own interphase by then. Failure of the micronucleus in *Paramecium* to express itself genetically (19, 20) and in *Tetrahymena* to incorporate an RNA precursor (1) have signified that it may be inert in vegetative cells, although some evidence also has been obtained for micronuclear function in *Tetrahymena* (24). Organisms lacking micronuclei, but not those lacking macronuclei, often are able to survive.

Experiments with normally growing, amicronucleate *T. pyriformis* have indicated macronuclear *S* and *G*2 periods closely resembling those reported here. Thymidine incorporation by cells of strain HS, having a constant generation time of 3 hours and a short *G*1, occurred within the first half of interphase; *S*, therefore, was something less than 90 minutes, and *G*2 was about 90 minutes long (14). Photometric measurements of individually cultured strain H cells with variable generation times also indicated that while DNA synthesis sometimes began soon after fission, it was completed at least 80 minutes, and often longer, before the following division started (9). By contrast, Walker and Mitchison (23) estimated from photometric studies of strain W grown in mass culture that synthesis continued throughout most of interphase, but they noted that this conclusion would not be justified if the organisms contained different basic amounts of DNA (as is the case in strain H (9)).

Compared with normally growing cells, rather different relationships of macronuclear *S* and *G*2 have been found in temperature-cycled micronucleate *T. pyriformis* WH6 (mating type I, variety 1) (3). Beginning a half hour or so after the first synchronous division, macronuclear incorporation of $TDRH^3$ continued for nearly 2 hours, and was completed only 10 to 20 minutes before the second synchronous division began. Events related to the micronucleus, however (except, again, for a longer *S*), closely resembled those reported here for normal cells. At the end of temperature cycling the micronucleus has been observed to exhibit a predivision configuration, terminated by its separation about an hour later (3, 5). Incorporation of $TDRH^3$ began immediately, and the first synchronous cell division followed in about a half hour; synthesis then continued for an additional 70 minutes or so (3).

Resemblances in the life cycles of *Tetrahymena* with different nuclear constitutions and under different conditions of growth do not universally apply to ciliates, however, as illustrated by another widely studied representative, *Paramecium*. Photometric measurements of *P. caudatum* (23) and *P.*

aurelia (7, 8, 25) have indicated that macronuclear *and* micronuclear DNA syntheses occur in the latter part of cellular interphase. Although both types of nuclei in *P. aurelia* start synthesis at mid-interphase, macronuclear *S* is about 4 times as long as micronuclear *S*. As in *Tetrahymena*, micronucleus divides about a half hour earlier than macronucleus. These ciliates, thus, differ not only in the times at which their nuclei initiate synthesis, but also in the relative intervals involved—*T. pyriformis* apparently requiring similar periods for both types of nuclei, and *P. aurelia* requiring a much longer period for the macronucleus (possibly related to the extreme degree of ploidy, 430 times that of a micronucleus (25)).

Since function of the micronucleus during vegetative growth is questionable, the time at which it synthesizes DNA (established for different ciliates, perhaps, early in their evolutionary history) may be of little consequence to the life of the cell. As long as the micronucleus maintains chromosomal integrity, it should be prepared for active participation in the events of conjugation (19). Possibly, however, its mitosis in vegetative cells might have some influence over fission times. For micronucleate *T. pyriformis*, an extended prometaphase has been found to precede micronuclear division. Instead of occupying a definite interval in normally growing cells, this period might, indeed, commence at the end of macronuclear DNA synthesis, and so influence the length of macronuclear *G*2 (conversely, if the *Tetrahymena* macronucleus similarly undergoes prolonged preparations for division, this might help to account for the duration of *G*2). A lengthy micronuclear prometaphase also required in *P. aurelia* (7), proceeding while the macronucleus completed synthesis, might "permit" a shorter macronuclear *G*2 than would otherwise be possible.

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