Antimitotic Agents and Macronuclear Division of Ciliates. III. The Effect of Colchicine on Synchronized *Tetrahymena pyriformis* GL

The alkaloid colchicine is known to exert an inhibitory effect on eumitotic cells by arresting mitosis at metaphase. According to SHELANSKI and TAYLOR[1] and BORISY and TAYLOR[2,3], colchicine seems to act directly on the protein-subunits of microtubules, including those of the spindle.

Recently, the occurrence of microtubular structures in macronuclei has been reported for a great many ciliates[4–8], among them the *Tetrahymena pyriformis* amicronucleate strain GL[9–12]. Such microtubules seem to be found predominantly in dividing macronuclei[9,12] and appear to be connected with the inner membrane of the nuclear envelope as well as with the chromatin material[9].

The division of macronuclei is generally referred to as 'amitotic' which is thought of as a simple pinching into 2 daughter nuclei without any ordered distribution of the chromatin. Since colchicine markedly affects the macronuclear division in *T. pyriformis* GL[13,14], it appears to be a reasonable assumption that such microtubular structures within the macronucleus could be functionally equivalent to the spindle microtubules in eumitotic cells.

Materials and methods. Logarithmic cultures of *T. pyriformis* amicronucleate strain GL were synchronized by employing the heat-shock method of SCHERBAUM and ZEUTHEN[15], as previously described[16].

Colchicine[17] (SERVA, Heidelberg, Germany) in a dose of 5 mg/ml was applied at different times after the end of heat-shock treatment (EHT). This concentration of the alkaloid is known to prevent division in an exponentially growing culture of *Tetrahymena* for about 5 h[13]. The drug was continuously present throughout the whole experiment. While the results described below could be reproduced 3 times for the drug concentration of 5 mg/ml, experiments employing lower concentration, e.g. 1.5 mg/ml, did not yield such reproducible data.

Results. Using the heat-shock method, the cell cycle of *T. pyriformis* GL can be brought into high division synchrony[15]. The first division maximum occurs with a yield of $80 \pm 5\%$ synchronization (i.e. a division index of 0.8) at 75 ± 5 min after EHT in our system. A second and third division peak of a lower degree of synchrony can be observed at 175 ± 10 min and 290 ± 10 min, respectively, after EHT (Figure 1a).

When colchicine was applied at EHT, the first division burst is delayed about 70 ± 10 min (Figure 1b). Also, the number of dividing cells is reduced to about 65%. The second peak then coincides with the third peak of the untreated control culture. Application of colchicine 35 min after EHT causes a marked delay of the first synchronization burst which occurs about 190 ± 15 min after EHT (D.I. ~ 0.55), followed 135 ± 15 min later by another less pronounced division peak (Figure 1c). But in 1 of 3 experiments a slight increase in the rate of dividing cells (D.I. $\leqq 0.1$) 80 min after EHT can be observed (Figure 1c). When cells were exposed to colchicine 45 min after EHT, the first division with a synchrony of about 55% is delayed only 15 ± 5 min (Figure 1d). A second peak can be detected 130 ± 15 min later. Colchicine added to the culture medium 55 min after EHT has no remarkable effect on the first division burst (Figure 1e). Only the right shoulder of this peak reveals a slighter decrease when compared with the corresponding maximum of the control. The second division peak can be observed at 260 ± 15 min after EHT, i.e. with a delay of 85 ± 15 min.

Apparently, there exists a 'transition point' 50–55 min following EHT where the preparation of cell events leading to division has matured so far that colchicine added from this moment cannot further bring about a 'set back' (Figure 2).

Discussion. Cell division of *Tetrahymena* can be blocked by colchicine and colcemid[13,14], i.e. alkaloids widely employed for arresting metaphase in eumitotic cells.

Surprisingly, *T. pyriformis* GL was found to overcome the inhibitory effect of such antimitotic drugs[13,14] after a period dependent on the concentration used, a phenomenon that had so far only been mentioned in the literature as occurring occasionally in regenerating liver[18] as well as in cultivated Chinese hamster cells[19]. This 'endogenous recovery'[13] results in a remarkably synchronized division peak[14]. As an explanation for this, we have tentatively discussed in a previous article the possibility that *Tetrahymena* possesses an enzyme system capable of decomposing colchicine and colchicine-related compounds, as this is known for some microorganisms[20].

The findings of blockage and delay of macronuclear division in *Tetrahymena* by colchicine as well as the occurrence of microtubules in the macronuclei of ciliates[4–6,8–10,12] shed some new light on the hypothesis[8,9] that these intranuclear microtubules play a role in the ciliate macronuclear division. This is all the more reasonable since it is hard to imagine a distribution of genetic material not involving distributing apparatus comparable to the mitotic spindle. Thus, according to the

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hypothesis by FALK et al.[9], the 'amitosis' of the ciliate would represent rather a special case of intranuclear mitosis.

This observation also is supported by the results of MAZIA and ZEUTHEN[21] who observed a blockage and a delay of cell division in heat-shock synchronized *Tetrahymena* by mercaptoethanol, an agent known to destroy spindle microtubules.

KENNEDY[22] states that he is unable to identify microtubules in the macronucleus as well as in the developing oral 'anlage' after application of colchicine. These findings are in agreement with our observations[23] that after treatment with colchicine no intact microtubules can be detected within the macronucleus. Microtubules, however, could normally be seen in the oral field, thus indicating a selective sensitivity of the different types of microtubules.

The existence of a 'transition point' at 50–55 min after EHT illustrates a moment in the cell cycle in which the preparation and stabilization of the compounds and structures required for division has proceeded so far that an attack by colchicine cannot further inhibit macronuclear and cell division. This could be due either to a change in the permeability of the drug to the structures in question or to a change in the stability of the microtubules concerned. The latter case then would represent a remarkable difference to eumitosis in which colchicine is active in inhibiting chromatin distribution up to metaphase and even to telophase[24].

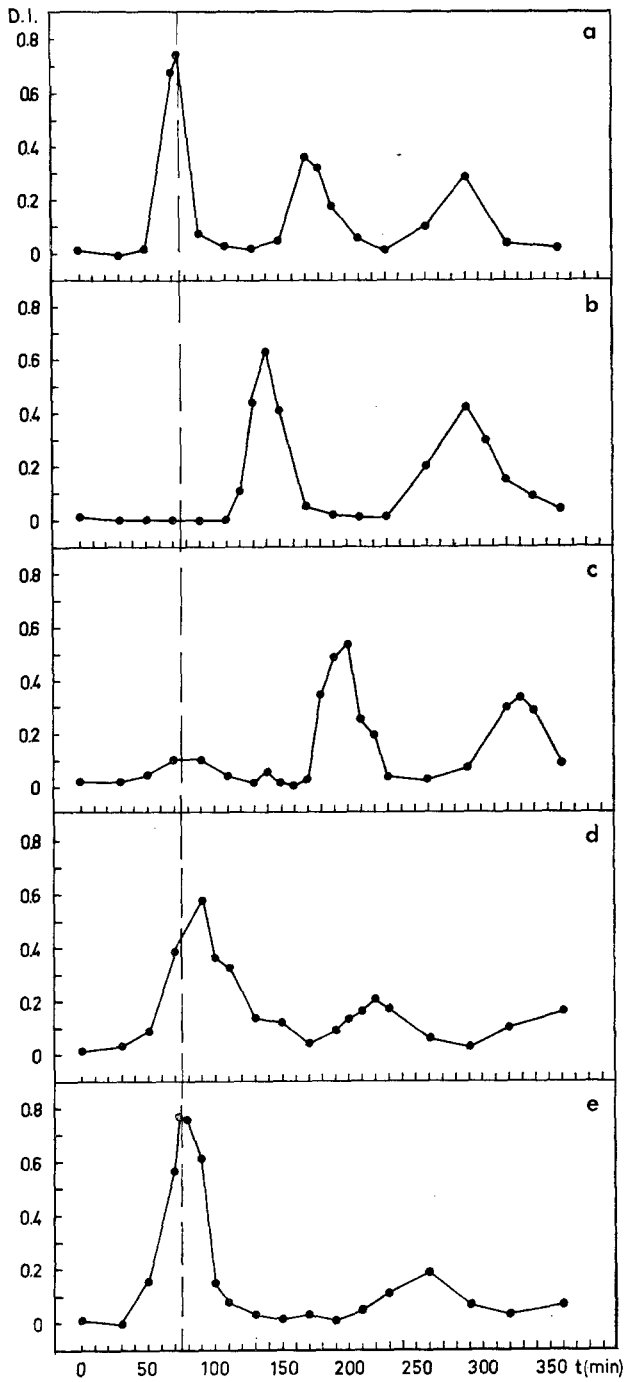

Fig. 1. Heat synchronized cultures of *T. pyriformis* GL were exposed to 5 mg/ml colchicine at different times after EHT. The division index of *Tetrahymena* is plotted against the time after EHT (EHT = zero time). (a) Untreated control culture. In (b), (c), (d) and (e) colchicine was applied at EHT, 35, 45 and 55 min after EHT, respectively. The vertical dotted line represents the average synchronized division peak of the untreated control culture.

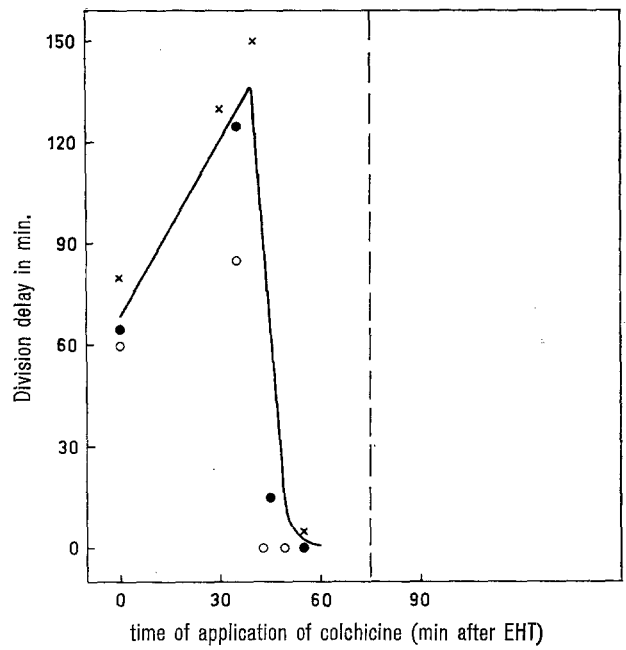

Fig. 2. The colchicine induced delay of the first division peak of heat synchronized *T. pyriformis* GL as dependent on the time of application of 5 mg/ml colchicine after EHT. Beyond a 'transition point' at 50–55 min after EHT colchicine does not further induce a 'set back' of the first division peak.

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The results described above include another interesting finding. The endogenous recovery from treatment with an antimitotic drug is essentially shortened in synchronized *Tetrahymena* cultures compared with logarithmic cells. This 'training effect' seems to be most reasonably explained by the assumption that both kinds of treatments, i.e. heat-shocks and colchicine, interfere with the very same compounds in the cell so that the heat-treatment brings about an 'adaptive change' (STUBBLEFIELD[19]) which helps to overcome the treatment with the drug[25,26].

Zusammenfassung. Durch Temperaturschocks synchronisierte Kulturen des Ciliaten *Tetrahymena pyriformis* (Stamm GL, kein Mikronucleus) wurden mit Colchicin behandelt. In Abhängigkeit vom Zeitpunkt der Colchicinzugabe wurden Verzögerungen des ersten synchronisierten Teilungsmaximums festgestellt. Die Ergebnisse werden als weitere Hinweise auf eine Beteiligung von Mikrotubuli bei der Makronucleus-Teilung gedeutet.

F. WUNDERLICH and D. PEYK

Department of Biology,
Division of Cell Biology, Schaenzlestrasse 9,
D-78 Freiburg i. Br. (W. Germany), 13 August 1969.

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[26] After the preparation of the manuscript the authors got knowledge of a paper by TAMURA et al. [Expl. Cell Res. *55*, 351 (1969)] in which a colchicine-induced disappearance of macronuclear microtubules was reported in *Tetrahymena pyriformis* strain W.

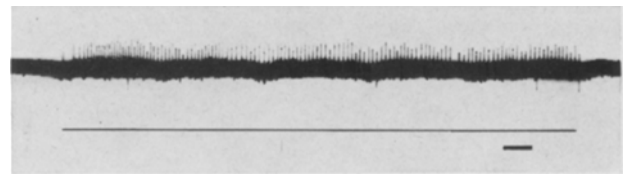
Trigeminal Root and Eye Muscle Proprioception

Proprioceptive impulses from the external eye muscles enter the brain stem through the ophthalmic branch and the trigeminal root in the lamb and pig[1,2]. The stretch of individual eye muscles provoked responses of units localized in the medial dorsolateral part of the semilunar ganglion and in the medial part of the trigeminal root. The responses were of the type induced by muscle spindle excitation[1,2]. Section of the ophthalmic branch abolished the gasserian responses to stretch of ipsilateral extraocular muscles[2,3]. However, no degeneration of the eye muscle spindles occurred after section of the ipsilateral oculomotor nerve[2,3]. The units recorded from the semilunar ganglion and responsive to stretch of single extraocular muscles were regarded as cells following the criteria proposed by DARIAN-SMITH et al.[4]. However, some investigators have claimed that the perikaria of the afferents from the eye muscles are placed in the mesencephalic nucleus of the trigeminus[5], as is the case for the masticatory muscles[5–10]. Thus, in order to state whether our previous records[1,2] were taken from cells or from nerve fibres, experiments were carried out in lambs subjected to chronic section of the left trigeminal root. Such an operation should abolish the gasserian responses to stretch of single extraocular muscles if the cell bodies of the afferents from the eye muscle spindles are placed in the brain stem, while, on the other hand, the responses should persist if the perikaria are located in the semilunar ganglion.

In 8 lambs both the sensory and the motor root of the left trigeminus were cut under Nembutal anaesthesia. The animals were kept alive 7–13 days in order to get complete degeneration of the nerve fibres and disappearance of nervous conduction in the peripheral trigeminal branches[11]. 6 lambs were submitted at the end of survival time to an acute experiment for searching the gasserian responses to stretch of individual extraocular muscles with the technique used in our previous investigations[1,2]; the other 2 animals were employed only for histological purposes.

In all the 6 lambs which underwent the final acute experiment, units were found in the left semilunar ganglion which responded to stretch of single eye muscles; the responses were of the type induced by muscle spindle excitation and exhibited the same features as those recorded from normal lambs (Figure). However, no responses of the type induced by muscle spindle excitation were found in the left semilunar ganglion by stretching the ipsilateral masseter in 2 animals.

The histological control showed normal spindles in the left extraocular muscles, while those of the left masseter were degenerated. No degenerated fibres were seen in the left ophthalmic branch; however, many degenerated fibres were present in the left mandibular branch.



Effect of a stretch of the left superior rectus (lower beam) on the unitary discharge (upper beam) recorded from the medial dorsolateral part of the left semilunar ganglion of lamb No. 77, 13 days after section of the ipsilateral trigeminal root. The units were unaffected by stretch of the other extraocular muscles and by stimulation of other trigeminal receptors. Calibration: 100 msec.

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