response to electrical stimulation. The second group had the following characteristics: 1. The voltage of the response was lower than the first group; 2. the time course of the rise of the evoked potential was slower than that of controls; 3. the voltage of the response was unstable and was made up of a number of peaks, the pattern of which was irregular; and 4. the amplitude of the response was labile and showed marked fatigue to repetitive stimulation.

Ganglia were subsequently subjected to pharmacological studies to establish whether or not 2 groups of ganglia could also be differentiated on the basis of their synaptic pharmacology. Some of these ganglia had the 4 characteristics of the novel electrical response listed above. In the latter preparations, repetitive stimulation (0.3 msec, 20 Hz, supramaximal, 30–60 min) of the splenic nerve abolished the ganglionic and the postganglionic responses to electrical stimulation and no restoration occurred with prolonged rest (up to 3 h). Complete restoration was accomplished, however, within 30 min after the addition of 25 ng/ml DL-3,4-dihydroxyphenylalanine (DOPA) to the bath medium (Figure 2A). Following a second stimulation period and rest period, complete restoration was seen in less than 8 min after the addition of a higher concentration of DOPA (125 ng/ml) (Figure 2B). DOPA by itself was only capable of restoring the abolished ganglionic response 2 or 3 times. Ganglionic transmission was thereafter restored only by the combined addition of DOPA and pyridoxal phosphate (pp, 0.05 m*M*) to the bath (Figure 2C). This DOPA-dependent ganglionic transmission was blocked by the ganglionic blocker, hexamethonium bromide (70 µg/ml) (Figure 2C).

In one of these 5 splenic reinnervated ganglia, no response was detected 2 h after isolation (normal recovery period). The addition of pp and DOPA to the bath yielded a ganglionic response to electrical stimulation within 10 min.

As a control, normal L_4 ganglia and self-reinnervated L_4 ganglia (reinnervated with its own preganglionic fibers from the sympathetic chain) were subjected to the same treatment. Following stimulation and recovery the ganglionic response showed at most a slight decrease in amplitude. Addition of pp alone caused a slight increase in the amplitude of the response, while DOPA alone or in combination with pp caused a decrease. Likewise, the compound action potentials of an isolated normal splenic nerve, showed a decreased amplitude after the addition of DOPA to the bath.

The data presented here suggest that when the L_4 sympathetic ganglion is reinnervated with fibers from the splenic nerve, new excitatory synapses are formed. In about half of the cases, the reinnervated ganglion shows normal electrophysiological responses, probably due to the presence of cholinergic fibers. The novel electrophysiological response of the other reinnervated ganglia and the dependence of their synaptic activation on the presence of DOPA and pp suggests that these sympathetic neurons may now have 'adrenergic' preganglionic fibers. Until further studies are done, however, a cholinergic component cannot be ruled out nor can the hexamethonium sensitivity be explicitly interpreted as an action of the monoaminergic transmitter on a nicotinic receptor. The sympathetic homoneurograft may provide a unique preparation for the analysis of sympathetic physiology and for synaptic plasticity[15].

Zusammenfassung. Sympathische Ganglien, wenn mit Fasern des *N. splenicus* reinnerviert, bilden 2 pharmakologisch differente Synapsengruppen: normale, vom cholinergischen Typ und ca. 20% exitatorische vom «monoaminergischen» Typ, wenn sie von adrenergen Fasern reinnerviert wurden.

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Synchronous Growth of *Polytomella agilis*

The growth characteristics of the flagellate protozoan, *Polytomella agilis*, have been studied in batch culture[1]. Under these conditions, cell volume, protein and carbohydrate decline during the logarithmic phase of growth. Thus, in spite of the constant rate of cell multiplication the growth of the population is unbalanced. From these observations, it is difficult to come to any meaningful conclusions about the description or regulation of growth of single cells in the population. Therefore, cultures of this organism were synchronized by means of a repetitive temperature cycle, using the rationale of James[2].

Cells were grown in 500 ml Erlenmeyer flasks in a complex medium consisting of 0.2% (w/v) tryptone, 0.1% (w/v) yeast extract and 0.2% (w/v) sodium acetate. Cell counts were made periodically using a hemocytometer. It was found that a cycle of 22 h at 9 °C followed by 2 h at 25 °C resulted in the complete doubling of the population during the warm period. This is in contrast to the mean generation times of this organism in batch cultures grown at these temperatures, which are 32 and 4.7 h respectively.

Figure 1 illustrates the synchronous growth of a population maintained on this repetitive temperature cycle. In addition to the change in cell number, the change in the division index (the proportion of cells undergoing division at any time) from 0.2% during the cold period to between 0.8% and 1.5% of the population

[1] P. Sheeler, M. Cantor and J. Moore, Protoplasma *69*, 171 (1970).

[2] T. W. James, in *Synchrony in Cell Division and Growth* (Ed. E. Zeuthen; John Wiley and Sons, Inc., New York 1964), p. 323.

during the warm period is indicated. Using a modified form of an equation derived by COOK and JAMES[3], it is possible to estimate the fission time of cells, namely

$$FT = \frac{\omega fn\ (DI + 1)\ DT}{\omega fn\ 2}$$

where FT is the time required for a cell to divide, DI is the division index and DT is the doubling time of the population. For a doubling time of 2 h, the fission time is calculated to be approximately 1.4–1.8 min. This estimate is verified by visual observation.

The biosynthetic activities of the cells are confined to the nondividing portion of the cell cycle. Cellular protein, analyzed by the method of LOWRY et al.[4], and RNA, estimated with DNA by a modified SCHMIDT-THANNHAUSER[5] technique, almost double during the cold period (Figures 2 and 3). Cellular DNA increases more than 3-fold during the same portion of the cycle (Figure 3). The coupling of RNA and protein biosynthetic rates is consistent with the notion of balanced growth under conditions of synchronous growth. The marked decrease in the DNA content during the warm period suggests that a major portion of this constituent is degraded into its component nucleotides at the time of division. During preparation, these molecules are extracted and consequently not measured. The increase in DNA during the nondividing portion of the cycle may be explained in 3 possible ways: 1. The chromosomes may be polytene. 2. There may be an increase in the ploidy of the cell. 3. There may be continuous DNA synthesis with the likelihood of several rounds of replication during the cold period. In any case, the data suggest that cell division in *P. agilis* is not triggered by the doubling of its DNA content.

Oxygen consumption over the entire cell cycle was measured by means of Warburg manometry. During the warm period and first 6 h of the cold period, the rates of oxygen consumption (Q_{O_2}) are 9.7 and 9.8 µl O_2/h/10^6 cells respectively. The Q_{O_2} doubles by 18 h in the cold period to 17.5 µl O_2/h/10^6 cells (see Table). It is not yet certain whether these data reflect a doubling of the mitochondria per cell or an increase in mitochondrial activity associated with increased cellular biosynthetic activity. Preliminary studies of whole cells and

Respiration of synchronized *Polytomella agilis*

Phase of cycle	Q_{O_2} (µl O_2/10^6 cells/h)
Warm period	9.71 ± 2.4 (9)
Cold period	
Early	9.84 ± 2.3 (11)
Late	17.57 ± 4.5 (9)

All values are given ± the standard deviation. The figures in parentheses indicate the number of experiments.

[3] J. R. COOK and T. W. JAMES, in *Synchrony in Cell Division and Growth* (Ed. E. ZEUTHEN; John Wiley and Sons, Inc., New York 1964), p. 485.
[4] O. H. LOWRY, N. J. ROSEBROUGH, A. K. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[5] G. SCHMIDT and S. J. THANNHAUSER, J. biol. Chem. *161*, 83 (1954).

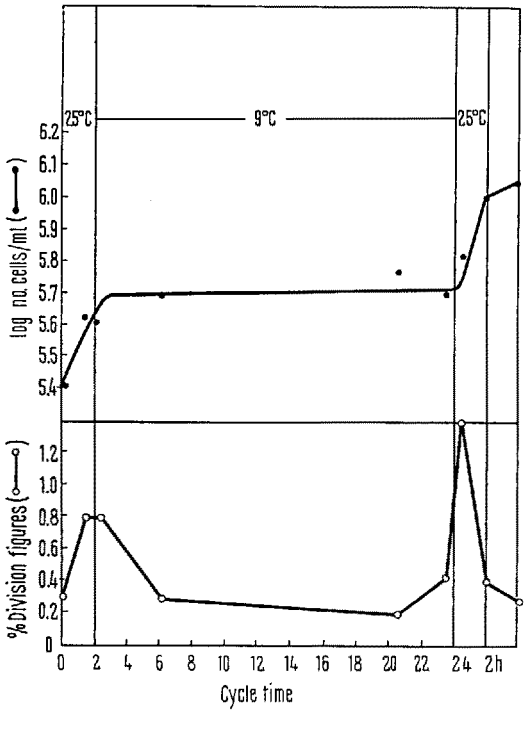

Fig. 1. Synchronous division of *Polytomella agilis*. The upper curve shows the cell number during the 2 h warm period (25 °C) and the 22 h cold period (9 °C). The lower curve indicates the division index (proportion of cells in fission) over the cell cycle.

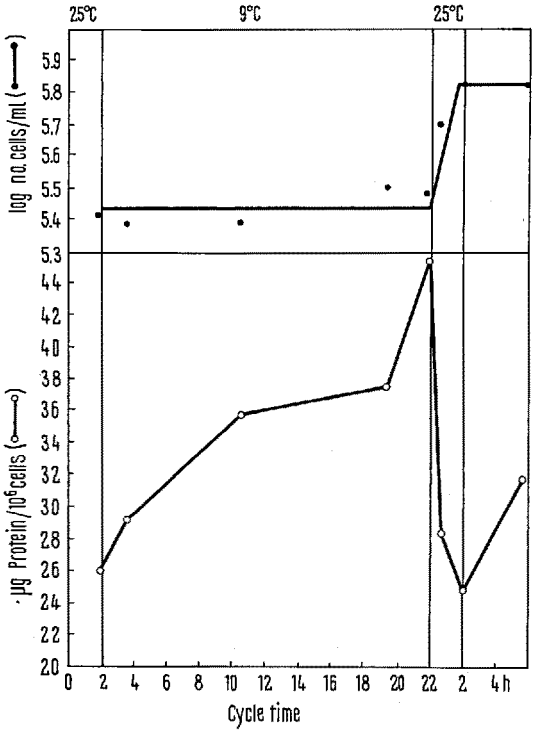

Fig. 2. Protein changes during the synchronous growth of *P. agilis*. The upper curve shows the changes in cell number over the cell cycle. The lower curve indicates the cellular protein content (µg/10^6 cells) sampled at the indicated times.

mitochondrial fractions indicate that there is a marked increase in respiration in the late logarithmic and early stationary phases of the growth curve when the rate of cell division decreases[6]. Metabolic activity becomes more pronounced in the absence of cell division.

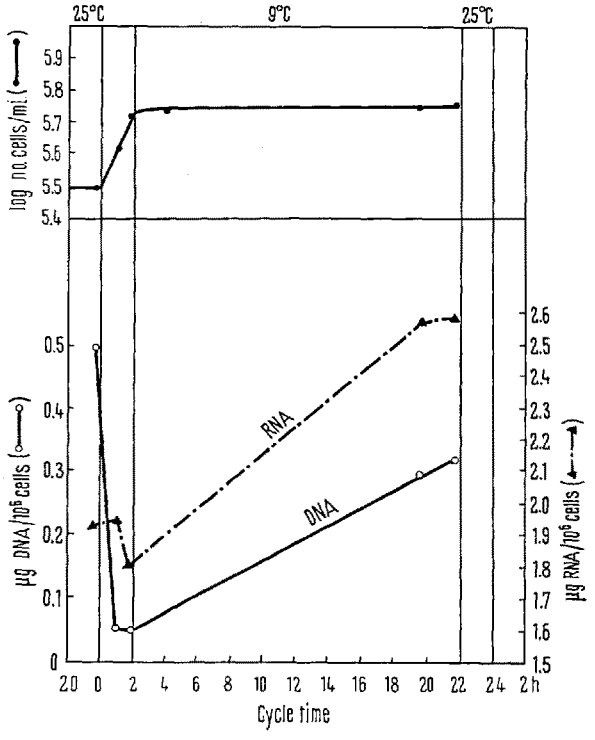

Fig. 3. Nucleic acid changes during the synchronous growth of *P. agilis*. The upper curve shows the changes in cell number over the cell cycle. The lower curve indicates the cellular RNA and DNA content of cells sampled at the indicated times. All values are expressed as $\mu g/10^6$ cells.

The use of synchronized populations of this organism will facilitate studies of other aspects of its physiology. *P. agilis* is capable of encystment in the course of its life cycle. Some of the biochemical and morphological changes associated with this process of cellular differentiation have already been described[7,8]. More precise study of the events occurring in encystment should be possible in synchronized populations. Metabolic adaptation for the utilization of propionate and butyrate as carbon sources for growth have been described in batch cultures[9]. The absence of balanced growth under these conditions render any attempt to specify control mechanisms inadequate. Synchronized populations will also be useful for the study of metabolic regulatory mechanisms in this organism.

Zusammenfassung. Das Wachstum des Infusors *Polytomella agilis* kann durch wiederholte Temperaturrhythmen von 22 h bei 9 °C und 2 h bei 25 °C synchronisiert werden. Verdoppelung der Zellpopulation findet in der Wärmeperiode statt und der Atmungsrate in der Kälteperiode, auf welche die Biosynthese beschränkt bleibt.

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Long Term Toxicity Studies with Endotoxoid in Monkeys

Changes in different parameters caused by endotoxins in experimental animals are essentially influenced by pretreatment with endotoxoid[1-3] a detoxified endotoxin from *S. marcescens*[4]. Considerable protection against lethal doses of X-irradiation is achieved after a single intravenous application of endotoxoid in mice[2,5]. A certain protection also was observed after intraperitoneal administration[6].

In vitalmicroscopical observations the severe disturbances of the capillary bed following injection of endotoxins fail to appear after pretreatment with endotoxoid[2,7]. Moreover, animals survive lethal doses of endotoxins from different gram-negative bacteria after a single injection of endotoxoid[1-3], thus inducing nonspecific endotoxin tolerance. The clinical aspects of these results were pointed out[2]. Related studies in human volunteers revealed that endotoxin-induced alterations are mitigated by pretreatment with endotoxoid[8,9].

In addition to extensive experiments in various species, clinico-therapeutic research with endotoxoid in humans implicates long term toxicity studies with this substance in primates. The objective of these experiments was to study the effect of endotoxoid on the function mainly of the liver, kidneys and the hematopoietic system with clinico-chemical parameters as well as the effect on all organs determined by necropsy and histologic evaluation.

In connection with these studies it should be mentioned that lately the hazard of performing experiments in monkeys has been recognized. A review of infections with *Herpes simiae* virus in the United States and Great Britain transmitted by the laboratory monkeys *Macaca mulatta* and *Macaca philipinensis* was published in 1960[10]. In 1967 an unknown virus, later referred to as Marburg virus[11-13], caused an epidemic in Germany and Yugoslavia infecting 31 persons among laboratory and clinic personnel, of whom 7 did not survive. All the infected persons had been in contact with *cercopithecus aethiops* or with material from these animals[11,14-17].

Herpes simiae only produces a mitigated clinical picture in monkeys similar to that of *Herpes hominis* in man, so that usually it is not recognized, whereas the infection with the Marburg virus is fatal also to monkeys. The largest experimentally observed time between inoculation with the Marburg virus and death has been 25 days in monkeys[18,19]. Therefore strict quarantine of at least 8 weeks should be compulsory to all monkeys prior to their