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.

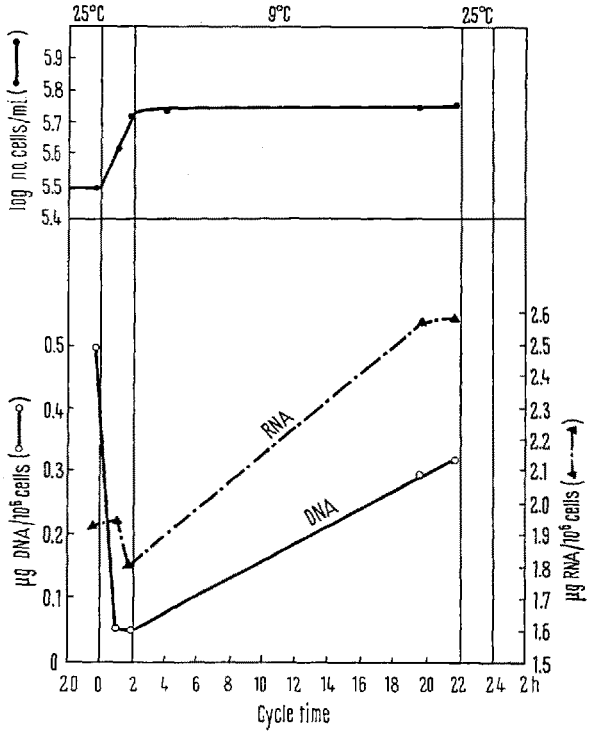

Fig. 3. Nucleic acid changes during the synchronous growth of *P. agilis*. The upper curve shows the changes in cell number over the cell cycle. The lower curve indicates the cellular RNA and DNA content of cells sampled at the indicated times. All values are expressed as $\mu g/10^6$ cells.

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

experimental use. There is no doubt about the danger of *Herpes simiae* virus and Marburg virus to humans, and although now diagnosis is possible to a satisfactory extent, therapy merely remains symptomatic and without prediction regarding the outcome[10,15,17]. Nevertheless it should be emphasized that the greatest risk may arise from infectious agents so far unknown, as has been pointed out before[13]. These agents could endanger also major sections of the population through vaccine via tissue cultures of kidneys from monkeys. Consequently the only possible safety precaution will be to regard monkeys and their material as potentially infected for an initial period of observation, the duration of which can hardly be determined if an unknown agent is to be considered.

The events mentioned led to a ministerial order by the Federal Government in Germany in 1967 only permitting the import of monkeys in exceptional cases. In addition the Federal Ministry of Health and a special Government commission[20] not only established obligatory quarantine for all monkeys but also regulated the keeping and experimental procedure with these animals. Similar safety measures have been described in detail[10] and the danger has been pointed out[13].

Methods. In the present study 10 *Macaca mulatta* of both sexes weighing 2.0–3.0 kg were used. The animals were kept according to the above-mentioned regulations. They were observed for an initial period lasting 4 weeks, after having been quarantined for 12 weeks in Great Britain. All monkeys were tuberculin negative and appeared healthy during the period of observation. Their chest X-rays did not reveal any pathologic changes. They received anthelmintic treatment and their faeces did not show any sign of contamination with *Salmonellae* and *Shigellae*.

Six animals received i.v. injections (vena saphena) of endotoxoid (charge Mex 25) 100 µg per kg of body weight diluted in 0.02 ml of sterile pyrogen-free physiological saline per kg. The dosage was given for the first 5 weeks 3 times a week and then increased to 5 times a week until the 13th week. 4 control animals received i.v. injections of 0.02 ml sterile pyrogen-free physiological saline per kg at the same time. Body weight and body temperature were recorded weekly.

Blood samples were obtained by saphenal venipuncture before the initial dosage and during the 4th, 8th and 12th week. The following parameters were determined: erythrocyte count, total and differential leucocyte counts, hematocrit (capillary method) and prothrombin time (Quick). Serum levels were analyzed photometrically for glucose (hexokinase-method) urea and bilirubin (colorimetric method) as well as for the following enzymes: alkaline phosphatase (colorimetric), glutamate dehydrogenase, glutamic-oxalacetic and glutamic-pyruvic transaminases by UV-method. Hemoglobin (according to DRABKIN) and the total protein content (biuret) were also determined. Commercially available reagents from Boehringer, Mannheim, were used for all tests. Serum proteins were analyzed immuno-electrophoretically and electrophoretically on cellulose acetate membranes.

Urine samples were collected at corresponding times in order to determine glucose, protein, hemoglobin levels (photometrically as described) and the presence or absence of urobilin and urobilinogen. Specific gravity, in terms of osmolality, and pH were measured; the sediment was examined microscopically.

Following the series of injections, some parameters of the blood coagulation system were also examined with reagents of Behringwerke: thrombin time, PTT, factor V, prothrombin, fibrinogen and thrombocyte count.

Two weeks after administration of the last dose all monkeys were euthanized with sodium pentobarbital (50 mg/kg). Gross and microscopic examination of all organs was carried out.

Results. Throughout the experiment the animals appeared healthy and no febrile response was evident. No essential changes occurred in the parameters measured as shown in Table I of this and the following paper.

The mean values of the electrophoretic analysis at the beginning of the experiment were: albumin 66%, α_1-globulin 2%, α_2-globulin 7%, β-globulin 10%, γ-globulin 15%. No significant changes in the serum protein pherograms or in the immuno-electrophoretic pattern were observed during the entire course of the experiment.

None of the urine tests mentioned showed any pathologic change and no difference between treated and control animals was seen.

The results of the determined parameters of the blood coagulation system are summarized in Table II.

The determination of parameters of the blood coagulation system, listed in Table II, showed no essential differences between controls and treated monkeys.

All animals showed low anti-endotoxoid titers in their sera prior to the injections as demonstrated using endotoxoid-coated human red blood cells in the passive hemagglutination test. Sera from untreated *Macaca mulatta*, which were kindly supplied by the Robert-Koch-Institute, Berlin, showed similar low titers. Uncoated carrier erythrocytes serving as antigen controls were negative. Thus all the *Macaca mulatta* we have examined exhibited hetero-antibodies which react with human erythrocytes coated with endotoxoid derived from *S. marcescens*. 2 weeks after the last injection the

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Table I. Mean values of results obtained in *Macaca mulatta* after i.v. application of endotoxoid

	Before injection Treated	Controls	4 weeks after first injection Treated	Controls	8 weeks after first injection Treated	Controls	12 weeks after first injection Treated	Controls
Body temperature °C	39.8	39.6	39.8	39.5	39.3	39.1	39.6	39.3
Bilirubin mg/100 ml	1.78	1.80	1.58	1.62	1.99	2.05	1.78	2.02
Alk. phosphatase mU/ml	277	272	207	300	269	300	248	289
SGOT mU/ml	31.10	24.93	34.90	31.43	25.17	23.3	31.32	26.91
SGPT mU/ml	21.42	19.28	22.45	23.28	14.45	12.84	23.02	25.29
GLDH mU/ml	3.61	2.71	3.42	3.52	3.24	3.88	3.37	3.33
Urea mg/100 ml	26.6	26.1	37.3	34.6	45.3	36.3	34.3	35.1
Glucose mg/100 ml	85.8	92.5	86.4	91.1	91.9	94.8	91.0	88.9
Protein g/100 ml	9.19	9.91	9.21	9.52	10.15	11.0	10.05	9.87
Prothrombin time (%)	80	89	82	81	81	85	81	89
Hematocrit (%)	49	46	48	48	46	45	47	47
Hemoglobin (%)	55.4	51.5	53.7	49.6	49.5	48.4	49.7	48.8
Erythrocytes mill/mm³	7.21	5.94	6.70	6.0	5.56	5.97	6.16	6.78
Leucocytes mm³	11,858	11,275	10,858	7,438	12,467	11,313	11,675	13,738

Table II. Mean values of blood coagulation studies determined at the end of the experiment

	Macaca mulatta	
	Treated	Untreated
Thrombin time/sec	27.5	22.1
PTT/sec	39.7	43.4
Factor V/sec	36.9	39.5
Prothrombin/sec	37.5	37.1
Thrombocytes/mm³	265,000	296,000
Fibrinogen	249	227

titers slightly increased in monkeys which had received endotoxoid whereas the titers of control animals remained unchanged. With the serological tests employed endotoxoid did not appear to evoke a good antibody response.

Macroscopic observation at necropsy did not reveal any alterations except for pleural adhesions and some little white nodes present in the lungs of one animal of either group. Inflammatory infiltrates in the area of the white nodes especially with eosinophile leucocytes and fibrous tissue obliteration (histiocytes and collagenous fibres) were microscopically observed[21], but obviously had no connection with the endotoxoid treatment[27].

Zusammenfassung. Vor dem Hintergrund der vielfältigen Endotoxoidwirkung wurde diese Substanz in chronischen Toxizitätsstudien bei *Macaca mulatta* geprüft. Endotoxoid wurde *Macaca mulatta* in einer Dosierung von jeweils 100 γ/kg in insgesamt 51 Injektionen innerhalb von 12 Wochen injiziert. Dabei zeigte sich, dass diese Substanz keinen Einfluss auf die geprüften klinisch-chemischen Parameter hatte und bei makroskopischer und histologischer Untersuchung keine Veränderungen auftraten.

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The Effects of Cyclophosphamide and Nor-Nitrogen Mustard Administration to One-Day-Old Mice

Cyclophosphamide is an antineoplastic agent that can produce abnormal development in both embryonic[1] and neonatal mice[2]. Bioactivation of the parent compound is required for the production of alkylating metabolites[3]. It has been suggested that the teratogenic effects of cyclophosphamide are associated with the parent compound rather than alkylating metabolites[4]. We have shown that the developmental toxicity of this drug, in perinatal mice, occurred at a time when the affected organism had not fully developed an in vitro ability to activate the parent compound[5]. However, since 1-day-old mice had a slight capacity to perform this conversion it became necessary to determine the effects of alkylating activity on neonatal development. If perinatal toxicity arises from an alkylating mechanism then other alkylating agents may produce similar effects. This communication reports the results of experiments in which cyclophosphamide and nor-nitrogen mustard, an agent with in vitro alkylating activity, were administered to 1-day-old mice.