Cytological and Cytogenetical Studies on *Paramecium Polycaryum*, III. Disturbance of the Conjugation Process of the Nucleus following the Application of Mitomycin-C[1)]

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(With 3 Tables and 1 Plate)

Effects of Mitomycin on mammalian cells *in vitro* were studied by Kobayashi (1959) who reported irregular coagulation of chromatin materials and nuclear fragmentation. The effect of this drug in protozoan cells of *Paramecium polycaryum*, was studied by Takayanagi (1960) with special reference to the nuclear apparatus. He observed a depression of the micronuclear number in individual paramecia and a deformation of the macronucleus following the treatment at vegetative phases. In the present study the effect of this drug on the nucleus was observed in the course of conjugation in *Paramecium polycaryum*.

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Material and methods: *Paramecium polycaryum*, collected from foul water in Sapporo City, was cultivated in test-tubes with a standard autoclaved lettuce medium consisting of 1.5 g desiccated lettuce and 1 liter deionized water to which the bacterium, *Aerobacter aerogenes*, was added as the food source. The culture medium was regulated with phosphate buffer at pH 7.0. Cultures were maintained by continuous serial transfers every 5–6 days. Four-day-old cultures grown at 21°C were normally used for mating mixtures.

For nuclear preparations, the following techniques were employed. The specimens were pipetted onto cover slips and fixed in Bouin's solution for five minutes. The fixative was removed by suction. The specimens were slightly dried to affix them to the glass surface, and transferred to 70 per cent alcohol to which saturated lithium carbonate had been adequately added. Then, cover slips were stored in 70 per cent alcohol. For the Feulgen reaction the samples were hydrolyzed in 1 N HCl at 60°C for 10 minutes and exposed to the Schiff reagent for two hours.

Concentrations of Mitomycin-C used here were 500γ and 1000γ per ml. A number of conjugating pairs were exposed to the agent during a period from the 1st prezygotic

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division (crescent stage) to the formation of nuclear anlagen involving the following three phases : (1) the whole conjugation process, (2) the period from the crescent stage to the formation of syncaryon, and (3) the period from syncaryon stage to the formation of nuclear anlagen.

Effects of Mitomycin-C

1. Observations through the whole conjugation process : Four hours after the animals of one mating type were mixed with those of the opposite type, the conjugation process usually shows crescent stage. At this stage, the conjugants were transferred to the experimental medium containing 500 γ per ml of Mitomycin-C and exposed up to the stage when the nuclear anlagen were completed. The results are given in Table 1. In both normal and treated mates, a syncaryon caused by cross-fertilization was observed approximately 12 hours after mating reaction. From then on, the treated conjugants showed a gradual retardation of nuclear division. The postzygotic division occurred about 1 to 2 hours later in treated

Table 1. Disturbance and time delay of postzygotic division after Mitomycin-C treatment in the whole conjugation process. *Numerator designates macronuclear anlagen, denominator indicates micronuclear anglagen.

Stage		Syn-caryon	Ist post. div.	2nd pots. div.			Ex-conj.			3rd post. div.				Anlage*						
No. of nuclei and anlagen		I	II	IV	III	II	IV	III	II	VIII	VII	VI	V	IV / IV	IV / III	IV / II	IV / I	III / IV	III / II	II / III
% of nuclei and anlagen		100	98	41	52	7	34	58	8	29	5	53	13	16	3	69	8	1	2	1
Hours after mating	untreat.	12	14	14			15			15				17						
	treat.	12	15	16			17			17				21						

conjugants than in untreated ones. The treated conjugants showed anlagen stage 4 hours later than the untreated conjugants. Variation in number of nuclei produced by the postzygotic division was also found after this treatment. In four-nucleate stage (Fig. 3) at completion of the 2nd postzygotic division, the number of nuclei in treated mates was three or four showing nearly equal ratio in occurrence (Table 1, Figs. 1 and 2). In eight-nucleate stage when the 3rd postzygotic division was completed, the number of nuclei varied ranging form 5 to 8 (Figs. 4–6), six-nuclei being most frequent (53 per cent). Two hours after the 3rd postzygotic division, four macronuclear anlagen and four micronuclear anlagen were formed in normal exconjugants, while the majority of treated organisms had four macronuclear anlagen, and micronuclear anlagen in quite varying numbers ranging from 1 to 4 (Figs. 7–9). Organisms having four macronuclear

anlagen and two micronuclear one were abundant in occurrence (69 per cent). The above feature may be closely correlated with the fact that the organisms containing six nuclei were observed in plenty in the eight-nucleate stage. It is of great interest from this experiment that the abnormal division of nuclei produced by the chemical treatment seems to correlate closely with the abnormality of micronuclear anlagen.

2. *Observations during the period from crescent to syncaryon stages*: The organisms in crescent stage of conjugation process were transferred to higher dosage of Mitomycin-C, 1000γ per ml in concentration. Most mates formed syncaryon approximately 12 hours after mating reaction. At this stage they were removed from the experimental medium to a normal one. In this condition the process of conjugation was observed in twenty exconjugants derived from ten mates. The results of observations are shown in Table 2. It was observed that the majority of exconjugants passed normally through later stages of nuclear development, and that four out of eight products grew to differentiate into macronuclear anlagen, while the rest differentiated into micronuclear anlagen. Only three cells indicated, as shown in Table 2, an abnormality in differentiation of micronuclei in contrast to the formation of macronuclear anlagen. Considered from the results of this experiment, it seems apparent that the agent applied in crescent to syncaryon stages failed to affect most of the organisms.

Table 2. Number of organisms showing various combination of macronuclear and micronuclear anlagen after Mitomycin-C treatment during from crescent to syncaryon stages.

	Number of anlagen		
	IV/IV	IV/III	IV/II
Number of cells observed	17	1	2

3. *Observations during the period from syncaryon to anlage stages*: Ten mates were exposed to Mitomycin-C solution at a concentration of 1000γ per ml at their syncaryon stage. Nuclear conditions were observed at the stage when the

Table 3. Number of organisms showing various combination of macronuclear and micronuclear anlagen after Mitomycin-C treatment during from syncaryon to anlage stages.

	Number of anlagen			
	IV/IV	IV/III	IV/II	IV/I
Number of cells observed	5	2	11	2

nuclear anlagen were formed. As shown in Table 3, it was observed that the number of micronuclear anlagen decreased exhibiting a variation ranging from 1 to 3, while four macronuclear anlagen were formed normally in the processes of nuclear development in most organisms. The results obtained in this experiment are almost identical with those derived from the observations of the whole conjugation process.

Discussion

Since the discovery of conjugation in *Paramecium polycaryum* (Diller 1958) and the determination of mating types (Hayashi and Takayanagi 1961), the behavior of the nuclear elements in the course of conjugation have become clear to a considerable extent. In this species as well as in other Paramecidae, all of the products derived from a syncaryon in the course of nuclear behavior seem to be wholly alike until the stage when the differentiation into two types, macro- and micronuclear anlagen occurs. Normally, the organisms have two-nucleate stages in completion of the 1st postzygotic division, four-nucleate stage in completion of the 2nd postzygotic division, and eight-nucleate stage in completion of the 3rd postzygotic division. Three postzygotic division follow, so that four macronuclear anlagen and four micronuclear anlagen are formed.

In the present experiments, it was observed that the nuclei which resulted from three postzygotic divisions were more or less abnormal in number, except for the features observed in the 1st postzygotic division. Referring to the results of the present experiments, it is interesting to see that most organisms normally contained four macronuclear anlagen, but there were a few organisms which evidently contained four micronuclear anlagen. It is very probable that Mitomycin-C exerts such effect as to make the differentiation of micronuclear anlagen specifically abnormal. In the chemical treatment of the organisms during the period from crescent to syncaryon stages, the whole process of postzygotic division showed a slight abnormality (Table 2). It seems probable that Mitomycin-C applied before the syncaryon stage results in no harmful effect on the organisms. Organisms treated between syncaryon and anlage stages showed abnormality in more or less degree, so far as the number of products in the course of their postzygotic division is concerned. It is of interest that the results here obtained are nearly identical with those derived from the chemical treatment of organisms in the period of the whole conjugation process.

Woodruff and Spencer (1923) reported that *Paramecium polycaryum* is unstable in number of micronuclei. Recently, it has been shown that in normal clonal cultivation of this species, the micronuclei tend gradually to decrease in number (Hayashi and Takayanagi 1961). Takayanagi (1960) using some chemicals indicated that a sudden depression of micronuclear number occurred in vegetative cells. In striking contrast, the macronucleus of this species is strikingly stable in distribution. In the light of above feature, it is likely that the stability in distribution of the macronucleus stands in fair contrast to that of the micronucleus

in this species.

Up to date, the critical times when the products derived from a syncaryon happen to differentiate into the macro- and micronuclear elements has remained undetermined, although a visible differentiation has been observed under the microscope when macronuclear anlagen appeared as larger nuclei.

As reported in the foregoing pages, Mitomycin-C applied to conjugants of *P. polycaryum* affects firstly the micronuclear division following 1st postzygotic division, and then results in the abnormal formation of micronuclear anlagen. It is then apparent that the critical time of differentiation into the micro- and macronucleus seems to be in the stage when the 1st postzygotic division is completed. It follows that the fate of the macro- and micronucleus may be decided at the stage immediately after the 1st postzygotic division.

Summary

1. The present study deals with effects of Mitomycin-C on *Paramecium polycaryum* in various stages of conjugation.

2. The paramecia which were treated with this drug in the whole conjugation process and during the period from syncaryon stage to the formation of nuclear anlagen showed a variation in number of micronuclear anlagen from one to four, while the formation of macronuclear anlagen remained almost unaffected (Tables 1 and 3).

3. Those treated during the period from crescent stage to syncaryon stage showed no abnormality in the postzygotic division (Table 2).

4. In the light of the above results obtained in the present study, it seems probable that the critical time of differentiation into macro- and micronucleus may be in the stage when 1st postzygotic division is completed.

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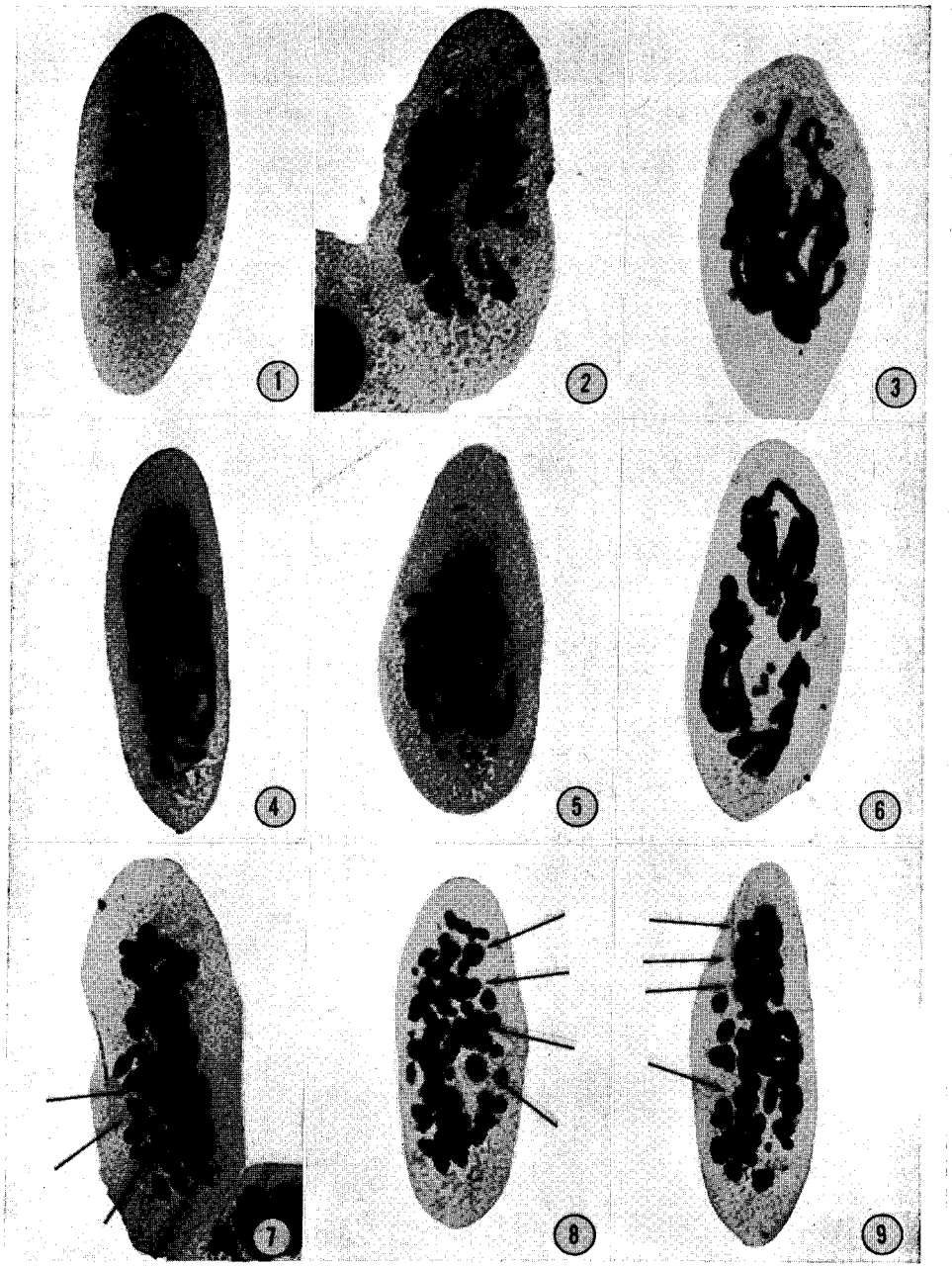
Explanation of Plate XV

Figs. 1–2. Treated cells which have three nuclei at the stage of 2nd postzygotic division. Fig. 1. The cell at the early stage of 2nd postzygotic division, × 480. Fig. 2. The cell at the later stage, × 480.

Fig. 3. Untreated cell which has four nuclei after completion of 2nd postzygotic division, × 480.

Figs. 4–6. Treated cells which have different number of nuclei at the stage of 3rd postzygotic division. Fig. 4. The cell contains five nuclei, × 480. Fig. 5. Six nuclei, × 480. Fig. 6. Seven nuclei, × 480.

Figs. 7–9. Treated cells which have four macronuclear anlagen and different number of micronuclear anlagen. Arrows show a macronclear anlagen. Fig. 7. A cell having single micronuclear anlage, ×480. Fig. 8. A cell having two micronuclear anlagen, × 480. Fig. 9. A cell having three micronuclear anlagen, × 480.



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