GENETIC STUDIES IN PARAMECIUM AURELIA

By

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</tbody>
</table>
Plate One. Paramecium cell stained with Feulgen. "Clouds" of mu particles seen around macronucleus.
Ma = macronucleus. Mu = Mu particles.

Plate Two. Mu particle after fixation in osmic acid as seen by electron microscope.

Plate Three. Mu particles from squashed paramecium viewed by phase contrast against an Indian ink background.
INTRODUCTION

Amongst the strains of Paramecium aurelia occurring in nature, a surprisingly high proportion have been found to contain the cytoplasmic particles called kappa, mu, pi, etc. (see Plate 1). Animals with these particles have been found to occur in varieties 1, 2, 4, 5, 6 and 8 of Paramecium aurelia. Since the particular varieties mentioned above have been found to occur in many different areas of the world, the presence of the particular cytoplasmic particles is by no means a rare discovery (Sonneborn, 1959).

The cytoplasmic particles occur in different strains in a diversity of shapes - some are rod-shaped, others spherical, and some even oblong. Recently two kinds of particles have been subjected to cytochemical and electron-microscopical studies. The particles termed kappa (Dippell, 1958, 1959) and those termed mu (Beale and Jurand, 1960) have been found to possess a complex structural organisation (see Plate 2). Both these cytoplasmic particles have a surrounding double membrane and appear to consist predominantly of deoxyribonucleic acid which is spread throughout the particle. The presence of some ribonucleic acid in both types of particle was also indicated. Each mu particle (but not kappa) was shown to have a capsule surrounding it (see Plate 3).

Paramecia containing particles of a given kind develop peculiar characteristics. When placed in contact with other paramecia lacking the particles, they bring about the death of the latter cells and are therefore called killers. The first killer paramecium was discovered in Variety 4 of Paramecium aurelia and its particles were kappa (Preer, 1950). The mode of killing was shown to involve the excretion into the medium of a substance or more complex agent which was toxic to animals lacking kappa
Plate Four. Mu particles from squashed paramecium seen by phase contrast microscopy.
particles. In a similar manner, animals from Variety 4 possessing lambda particles (several times larger than kappa particles) brought about the death of animals without lambda particles (Sonneborn et al, 1959). This killing effect, which was much more rapid than that of kappa, also involved the excretion of a toxic agent. On the other hand, studies in Variety 1 (Beale, 1957) and Variety 8 (Siegel, 1953) have revealed that the particles in certain stocks or strains of these varieties were associated with a different killer phenotype. These mu particles were found in the paramecia which behaved as mate-killers (see Plate 4). When mate-killer animals were made to conjugate with other stocks of Varieties 1 and 8, the latter mates—sensitives—died without dividing, or after one asexual fission. The ex-conjugant derived from the mate-killer animal survived and grew on to produce a clone of mate-killers, all containing mu particles.

The mate-killing effect differed from the form of killing associated with kappa and lambda, since it was dependent on prolonged contact between animals, which occurs during conjugation (Sonneborn, 1950) and not on excretion of a 'killer substance' into the medium. 'Mate-killing' was shown to be a surface phenomenon by the occasional occurrence both in Varieties 1 and 8 of three animals in prolonged contact. In such a case, two of the trio were found to die on separation, and one animal, the mate-killer animal, continued to divide asexually. Mate-killing was therefore not dependent on nuclear exchange, since exchange of nuclei could only take place between two of these three animals, the mate-killer and one of the doomed sensitive animals.

A striking feature of all these cytoplasmic particles was that they were each shown to be dependent on a certain gene or genes in the nucleus. Kappa particles were maintained in the cytoplasm of animals of Stock 51
(Variety 4) by gene K (Sonneborn, 1943) while recently the lambda particles of Stock 299 (Variety 8) which are associated with the rapid type of killing effect have been shown to be supported by a gene L (Sonneborn, Mueller & Schneller, 1959). The growth of mu particles in Stock 138 (Syngen 8) has been shown to be determined by a gene M, while we have shown in mate-killer Stock 540 (Variety 1) (Gibson & Beale, 1961) that the maintenance of mu particles is controlled by two dominant, unlinked genes M1 and M2. Either of these genes, however, was by itself sufficient for supporting the growth of the mu particles in the cytoplasm. When the genes K, L, M, M1 and M2 were replaced respectively by the recessive genes k, l, m, and simultaneously by m1 and m2 the appropriate particles were lost irreversibly from the cytoplasm of the paramecia. The particles were never found to re-appear even on the re-introduction of the original genes K, L, M, and both M1 and M2. In Variety 4 when cells of genotype Kk with kappa particles became kk after the appropriate cross, the particles remained present for at least eight fissions after the genotypic change. After the next few fissions a proportion of paramecia retained the particles, but eventually all were thought to lose them (Chao, 1953). Precise investigations of this sort had not been made with the other particles following the introduction of the recessive gene. In the work to be described here the events following simultaneous substitution of M1 and M2 by m1 and m2 in mate-killer Stock 540 (Variety 1) will be considered in detail.

Kappa was also shown to have another property. It was found possible to make a preparation of kappa particles which could be re-introduced into a sensitive cell of the genotype KK without any kappa particles. This sensitive cell was derived from a killer animal of genotype KK with kappa
EMIM particles, by environmental manipulation e.g. growth at high temperature. In the cytoplasm of this cell the infected kappa particles were maintained, multiplied to give large numbers, and the sensitive cell became a killer. However, it was found that infection of cells with genotype K K was not successful - the cells remained as sensitives (Sonneborn, 1959).

There has been a large amount of speculation concerning the origin and significance of these various particles. In face of all the evidence presented above and elsewhere, it is considered that the cytoplasmic particles are akin to bacteria. At some time in their history they are presumed to have been free-living but had invaded the cytoplasm of the paramecium. The evidence favouring this conclusion is as follows. The sizes of the particles are in the range of the larger bacteria; some at least of the particles (kappa) are able to be infected into other paramecia; the particles can be eliminated irreversibly from the cytoplasm and finally they have been shown to possess structural complexity. For instance, like some bacteria, mu particles have been shown to possess a capsule. On the other hand, the distribution of deoxyribonucleic acid throughout the kappa and mu particles is in contrast to the situation in commonly studied bacteria, where this substance is found in a central ("nuclear") body. This can perhaps be explained by assuming that in becoming adapted to their cytoplasmic environment the kappa and mu particles had become modified. In general the cytoplasmic particles called kappa, mu, lambda, etc., are considered to be symbionts.

The work to be described here concerns the relationship between the genes M_1 and M_2 and the mu particles in Variety 1 of Paramaecium aurelia. In one respect this is a study of the mechanism whereby the genes of the host are involved in the maintenance of symbiotic organisms. In a wider sense it is an investigation of how genes control cytoplasmic elements.
Perhaps most interesting of all, however, is the light these studies might throw on gene action and this is the subject of the work to be described in this thesis.
The various stocks of Variety 1 of *Paramecium aurelia* used are shown in the following Table.

<table>
<thead>
<tr>
<th>Stocks used</th>
<th>Mate-killer with mu particles or Sensitive without mu particles</th>
<th>Genotype</th>
<th>Derivation of Stocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>540</td>
<td>Mate-killer</td>
<td>m1M1m2M2</td>
<td>Originally collected in Mexico</td>
</tr>
<tr>
<td>540</td>
<td>Sensitive</td>
<td>m1M1m2M2</td>
<td>For preparation see below</td>
</tr>
<tr>
<td>513</td>
<td>Sensitive</td>
<td>m1M1m2M2</td>
<td>Originally collected in France</td>
</tr>
<tr>
<td>Tester 7</td>
<td>Mate-killer</td>
<td>m1M1m2M2</td>
<td>By crossing Stock 540 mate-killers to Stock 513 sensitives</td>
</tr>
<tr>
<td>Tester 5</td>
<td>Mate-killer</td>
<td>m1M1m2M2</td>
<td>By crossing Stock 540 mate-killers to Stock 513 sensitives</td>
</tr>
</tbody>
</table>
METHODS

1. **ELIMINATION OF MU PARTICLES FROM CYTOPLASM OF MATE-KILLER ANIMALS**

The following method was used to remove the mu particles from the cytoplasm of mate-killing paramecia.

A small culture (10-20 ml) of mate-killer animals with mu particles were placed in a 2 litre conical flask containing 1-2 litres of bacterised lettuce medium. After several days the paramecia had divided and used up the bacteria, with the result that the lettuce medium became clear. All the mate-killer cells, which had previously contained mu particles, were found to have lost them and become sensitives. This phenomenon was discovered by Dr. A. Jurand and has been found to be a highly successful method for removing mu particles. It seems probable that this effect is connected with the particular physiological conditions pertaining when there is a large excess of bacteria present in lettuce medium. The mu particles in Stock 540 can also be eliminated by growth at a high temperature (Beale, 1957).

2. **GROWTH OF PARAMECIA IN VARIOUS MEDIA**

The different clones of animals were maintained in lettuce medium at pH 6.4 in which one asexual fission occurred every eight hours at 25°C. Occasionally animals were placed in a phosphate buffer solution with the following constituents:

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium citrate</td>
<td>1M</td>
<td>20 ml</td>
</tr>
<tr>
<td>Sodium phosphate monobasic</td>
<td>1M</td>
<td>10 ml</td>
</tr>
<tr>
<td>Sodium phosphate dibasic</td>
<td>1M</td>
<td>10 ml</td>
</tr>
<tr>
<td>distilled water</td>
<td></td>
<td>945 ml</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>1M</td>
<td>15 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

In this medium the animals did not divide but usually remained alive and starved down for four days. However, occasionally when paramecia were placed in this medium, death occurred.
Plate Xv. Mu particles and bacteria as seen by the electron microscope.
Ba = Bacteria. F.V. = Food Vacuolo. Mu = Mu particle.
3. General methods connected with life cycle phases of Paramecium aurelia

In the experiments to be reported conjugation autogamy, cytoplasmic exchange, etc. were induced by the standard methods of Sonneborn (1951).

4. Examination of paramecia for mu particles

The main procedure in the following work was to discriminate between mate-killer and sensitive animals by scoring for the presence or absence of mu particles. Before scoring for mu particles animals were starved for a 24 hour period in the non-nutrient fluid (page 7). It was essential to carry out this period of starvation for two reasons:

(a) The bacteria present in the normal culture medium (aerogenes) were easily confused with the mu particles (see Plate 5). With a prior starvation period of 24 hours, however, the bacteria were largely eliminated due to digestion by the animals.

(b) Starvation also resulted in a large increase in the number of mu particles in each animal, thus rendering scoring for presence or absence of mu particles an easier task.

After starvation animals were squashed singly under a coverslip and the exudate examined under phase contrast. Previous work had shown that the presence of mu particles was always associated with the mate-killer phenotype, and mu particles were never found in sensitive cells. When particles similar to those shown below were seen the animal was scored as a mate-killer.

5. Method of replacing the dominant genes M₁, M₂, or both by their respective recessive alleles and isolating the required genotypic class

The object of the method reported here was to obtain a genotypic change involving the substitution of the genes M₁ and M₂ by their respective recessive alleles m₁ and m₂ in mate-killer animals. The mate-killer animals all had mu particles and were of three different genotypes:

(a) M₁m₁M₂m₂ (prepared from Tester 7 - see Fig. 1 and below)
(b) M₁m₁m₂m₂ (prepared from Tester 5)
(c) M₁m₁M₂m₂ (prepared from Stock 540)

The details of the gene substitution for situation (a) are shown in Fig. 1.
Figure 1. Method of preparation of clones of genotype $m_1 m_2 m_3 m_4$ from mate-killer clones of genotype $m_1 m_2 M_2 M_2$ (Tester 7). (See Text)
Tester 7 mate-killer animals (m₁m₁M₂M₂) with mu particles were crossed to sensitive Stock 513 without mu particles. The ex-conjugant derived cytoplasmically from each Tester 7 mate was now of genotype M₁m₁M₂M₂ and was grown on to produce an F₁ clone, each animal containing mu particles. The other mate derived from Stock 513 cytoplasm died as a result of mate-killing. The surviving F₁ clones were then either induced to pass through autogamy or else back-crossed to Stock 513 cells (m₁m₁M₂M₂).

After autogamy in the heterozygotes, clones of the two genotypes m₁m₁M₂M₂ and m₁m₁M₂M₂ were produced in equal proportions; after backcrossing, clones of genotype M₁m₁M₂M₂ and m₁m₁M₂M₂ were also produced in equal proportions as shown in Figure 1. Following autogamy or backcrossing the ex-autogamous or ex-conjugant individuals were allowed to divide once. One of the two daughter cells was placed in bacterised medium and allowed to divide about fifteen times. The sister cell was prevented from dividing by placing it in the non-nutrient culture fluid described in the "Methods" section. Some of the animals - about 20 - which were obtained after fifteen fissions from each isolate, were scored for the presence or absence of mu particles. If all the cells examined for mu particles had them, then this clone was classified as m₁m₁M₂M₂ or M₁m₁M₂M₂ and was discarded. Clones containing gene M₂ remained permanently as mate-killers with mu particles. If, however, cells without mu particles, i.e. sensitive cells, were present after the fifteenth fission, this clone was classified genotypically as m₁m₁M₂M₂ and the daughter animal at the 'one fission' stage after mating or autogamy which had been starved in the non-nutrient culture fluid in the same clone, was used for the rest of the experiment.
The details of analysis of the $m_{1}m_{1}m_{2}m_{2}$ clones, identified as described above, were as follows. In these clones the daughter cell which had been held back in non-nutrient fluid at the 'one fission' stage after autogamy or backcrossing, was placed in bacterised lettuce medium and allowed to pass through exactly six fissions, i.e. to the 'seventh fission' stage after conjugation or autogamy. After this number of fissions 64 cells were produced. Thirty two of these were taken at random, placed in separate depression slides and allowed to divide once more in lettuce medium. From here onwards, samples of 32 animals were picked out from the 64 obtained at each fission and the remainder were discarded. This procedure was carried out until a stage was reached, i.e. (number of fissions) at which it was required to score animals and to determine the proportion of mate-killer animals present. At the fission stages under study, 50 out of the 64 animals were taken, placed in non-nutrient culture fluid for 24 hours at $25^\circ C$ and allowed to starve down, as mentioned on page 8. Each group of 50 animals scored was derived from a separate ex-conjugant or ex-autogamous animal and was considered to comprise a separate clone.

Similar experiments were carried out by starting with Tester 5 mate-killer animals ($m_{1}m_{1}m_{2}m_{2}$) - situation (b) and Stock 540 mate-killers ($m_{1}m_{1}m_{2}m_{2}$) - situation (c). From these mate-killers, clones of genotype $m_{1}m_{1}m_{2}m_{2}$ were prepared and daughter animals scored for mu particles at the fissions following gene substitution.

6. Treatment of paramecia with ribonuclease and 8-azaguanine

During the experiments to be reported later, mate-killer paramecia were treated with ribonuclease (Light Co.) and with 8-azaguanine (Light Co.). One ml. portions of various concentrations of these two substances were
added to samples of one ml of mate-killer paramecium cultures containing a known number of animals. These substances were dissolved in 1 ml. portions of the non-nutrient solution whose constituents have been described in the "Methods" section and whose pH was adjusted to 7.1. The final concentrations of substances varied from .125 to .625 mg/ml ribonuclease and from .0125 to .05 mg/ml azaguanine.
Table 1. NUMBER OF CELLS WITHOUT MU PARTICLES AT VARIOUS STAGES FOLLOWING REPLACEMENT OF GENE $M_2$ by $m_2$ IN MATE-KILLER CELLS ($m_1m_1M_2m_1$)

<table>
<thead>
<tr>
<th>Number of fissions after change of genotype from $m_1m_1M_2m_2$ to $m_1m_1m_2m_2$</th>
<th>Number of paramecia examined for mu particles</th>
<th>Percentage of paramecia without mu particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>148</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td>10</td>
<td>256</td>
<td>32</td>
</tr>
<tr>
<td>11</td>
<td>100</td>
<td>61</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>13</td>
<td>100</td>
<td>79</td>
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<tr>
<td>14</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>93</td>
</tr>
</tbody>
</table>

Table 2. NUMBER OF CELLS WITHOUT MU PARTICLES DURING FISSIONS FOLLOWING REPLACEMENT OF GENE $M_1$ by $m_1$ IN MATE-KILLER CELLS ($m_1m_1m_2m_2$)

<table>
<thead>
<tr>
<th>Number of fissions after change of genotype from $m_1m_1m_2m_2$ to $m_1m_1m_2m_2$</th>
<th>Number of paramecia examined for mu particles</th>
<th>Percentage of paramecia without mu particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td>11</td>
<td>100</td>
<td>61</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>82</td>
</tr>
</tbody>
</table>
Table 2. NUMBER OF CELLS WITHOUT MU PARTICLES DURING FISSIIONS FOLLOWING REPLACEMENT OF GENES $M_1$ AND $M_2$ DURING THE SAME CONJUGATION BY $m_1$ AND $m_2$

<table>
<thead>
<tr>
<th>Number of fissions after change of genotype from $M_1 m_1 M_2 m_2$ to $m_1 m_1 m_2 m_2$</th>
<th>Number of cells containing mu particles</th>
<th>Percentage of paramecia without mu particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>29</td>
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<tr>
<td>11</td>
<td>100</td>
<td>34</td>
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<td>12</td>
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<td>13</td>
<td>102</td>
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<td>14</td>
<td>100</td>
<td>82</td>
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<tr>
<td>15</td>
<td>100</td>
<td>92</td>
</tr>
</tbody>
</table>
RESULTS

PART I

Section 1. Studies on loss of mu particles following the replacement of dominant genes \( M_1 \) and \( M_2 \) by the recessive alleles \( m_1 \) and \( m_2 \)

(a) Loss of mu particles following the replacement of the genes \( M_2 \) with \( m_2 \) in mate-killer animals of genotype \( m_1 M_2 m_2 m_2 \)

By appropriate crosses utilising Stock 513 the dominant gene \( M_2 \) in mate-killer animals of genotype \( m_1 m_1 M_2 m_2 \) containing mu particles was replaced by the recessive gene \( m_2 \). By the procedure outlined in the "Methods" section (page 10) animals were scored after fissions 8-15 following the genotypic change. In each clone fifty animals were scored for mu particles at these fission stages. The results of these experiments are shown in Table 1.

(b) Loss of mu particles following the replacement of the gene \( M_1 \) with \( m_1 \) in mate-killer animals of genotype \( M_1 m_1 m_2 m_2 \)

In the same way cells of genotype \( M_1 m_1 m_2 m_2 \) with mu particles were scored after fissions 8-15 following change of genotype to \( m_1 m_1 m_2 m_2 \). The results are shown in Table 2.

(c) Loss of mu particles following the simultaneous replacement of the genes \( M_1 \) and \( M_2 \) with \( m_1 \) and \( m_2 \) in mate-killer animals of genotype \( M_1 m_1 m_2 m_2 \)

The number of animals possessing mu particles in separate clones was also determined after fissions 8-15 after the simultaneous substitution of the genes \( M_1 \) and \( M_2 \) by their respective recessive alleles \( m_1 \) and \( m_2 \). The results of the experiments are shown in Table 3. The results of the experiments in sections (a), (b) and (c) are shown in graphic form below (Figure 2).

It will be seen from the Tables 1, 2 and the graph in figure 2, that similar percentages of paramecia lacking mu particles were obtained at the
Figure 2. Proportions of paramecia with and without mu particles after eight-fifteen fissions following loss of genes $M_1$ and $M_2$. 
tenth (32-33%) and eleventh (61%) fission stages following the replacement of either of the dominant genes $M_1$ or $M_2$ by their respective alleles $m_1$ or $m_2$. When counts of cells without mu particles were made at the 'twelfth fission' stage following substitution of one dominant gene by the recessive allele however, in the case of gene $M_2$ the percentage was found to be 71%, while the experiment with $M_1$ yielded a higher percentage - 82%.

The experiments involving the simultaneous replacement of the two dominant genes $M_1$ and $M_2$ by the recessives $m_1$ and $m_2$ yielded at particular fission stages percentages which were different from those obtained when only one dominant gene was replaced by a recessive allele. In the former case 77%, 64% and 34% were the percentages of sensitivities at the 'thirteenth, twelfth and eleventh fission' stages respectively, while in the latter case the percentages of sensitive cells were 79%, 71% and 61% respectively at these fissions, as can be seen from the graph in figure 2 and from the accompanying Tables.
Table 4. EFFECT OF STARVATION AND TEMPERATURE ON THE DISAPPEARANCE OF MU PARTICLES FOLLOWING CHANGE OF GENOTYPE FROM $m_1 m_1 m_2 m_2$ to $m_1 m_1 m_2 m_2$

<table>
<thead>
<tr>
<th>Time of starvation of animals one fission after genotype change (in hours)</th>
<th>Temperature during growth of cells ($m_1 m_1 m_2 m_2$)</th>
<th>Number of fissions between genotypic change of scoring for mu particles</th>
<th>Total number of animals scored</th>
<th>Percentage of animals lacking mu particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>18°C</td>
<td>12</td>
<td>102</td>
<td>74</td>
</tr>
<tr>
<td>72</td>
<td></td>
<td>12</td>
<td>102</td>
<td>71</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>12</td>
<td>98</td>
<td>73</td>
</tr>
<tr>
<td>72</td>
<td>25°C</td>
<td>12</td>
<td>102</td>
<td>72</td>
</tr>
<tr>
<td>96</td>
<td></td>
<td>11</td>
<td>50</td>
<td>60</td>
</tr>
</tbody>
</table>
Section 2. The influence of the environment on the rate of loss of mu particles following the replacement of the dominant gene $M_2$ by the recessive allele $m_2$

(a) Starvation of animals at the 'first fission' stage with subsequent growth at 18°C and 25°C

As already outlined in the "Methods" section, during the preparation of $M_1M_1M_2m_2$ clones there was a period of starvation of paramecia at the 'one fission' stage. Since this starvation period might influence the rate of loss of mu particles a series of experiments was carried out in which this period was varied. The times of starvation studied were 24 hours, 72 hours and 96 hours at 25°C. At this temperature the animals grew and divided once every six hours when in bacterised medium. After each starvation period the paramecia were allowed to pass through eleven asexual fissions and then scored for mu particles at what was effectively the 'twelfth fission' stage after gene replacement.

A further series of experiments was carried out with periods of starvation of 24 hours and 72 hours, but with ensuing growth at 18°C (one fission occurring every 12 hours). Once more after eleven further fissions (i.e. 12 in all), cells were scored for presence or absence of mu particles, i.e. at the 'twelfth fission' stage. The results of these experiments are shown in Table 4.

By comparing the percentages in the last column of Table 4 with the percentages at the appropriate (i.e. eleventh and twelfth) fission in Table 1, it will be clear that variation in starvation time of the 'first fission' stage cell and variation of the growth temperature had no effect on the rate of loss of the mu particles. For example, after 24 hours starvation of the 'first fission' cells and growth at 25°C, the percentage of cells at the 'twelfth fission' stage which lacked mu particles
Table 5. NUMBER OF PARAMECIA WITH AND WITHOUT MU PARTICLES FOLLOWING REPLACEMENT OF GENE M₀ BY m₀ WITH SUBSEQUENT GROWTH IN YEAST MEDIUM AT 25°C (WITHOUT STARVATION BEFORE SCORING)

<table>
<thead>
<tr>
<th>Number of fissions after genotypic change</th>
<th>Number of animals scored</th>
<th>Percentage of paramecia without mu particles</th>
<th>Percentage of cells with final starvation and growth in bacterised medium (see Table 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>50</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>94</td>
<td>93</td>
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</tbody>
</table>
was 72%, while 72 hours starvation and growth at 18°C resulted in 73% of the cells being sensitives at this same fission.

(b) Starvation of animals prior to scoring for mu particles
(see page 8)

In the "Methods" section it was pointed out that starvation of paramecia in non-nutrient fluid for 24 hours prior to scoring for mu particles was carried out for reasons pointed out there. In the experiments to be reported here the preparation of the $m_1m_2m_2$ clones was carried out in yeast medium thus eliminating the necessity to starve paramecia for 24 hours before scoring for mu particles. The division rate of cells in this yeast medium was 1½ fissions in 24 hours at 25°C. Paramecia were scored after eight, ten and fifteen fissions following the replacement of the dominant gene $M_2$ by the recessive allele $m_2$. Fifty animals were examined for mu particles in each clone at each fission, without any starvation period before scoring. The results of these experiments are shown in Table 5.

From Table 5 it will be seen that the loss of mu particles was at a similar rate either when the paramecia were grown in normal bacterised lettuce medium with a 24 hour period of starvation before scoring animals, or in yeast medium with no starvation before scoring. It was also clear from these experiments that the small proportion of mate-killer cells (7%) obtained as many as fifteen fissions after gene replacement in yeast medium had large numbers of mu particles (0.300 per animal).

To sum up, after genic substitution ($M_2$ by $m_2$ etc.) the mu particles remain present in most if not all daughter animals for at least seven fissions. From this stage on, some cells lacking particles (sensitives) appear abruptly after each fission until the fifteenth. The environmental conditions of starvation, temperature and growth rate did not alter the rate at which cells
Figure 3. Number of macronuclear fragments seen in each daughter animal at various fissions after conjugation.
lacking mu particles occurred.

Section 3. Behaviour of macronuclear fragments following conjugation of mate-killer animals with sensitive animals

(a) Without starvation of ex-conjugants

Since the existence of the macronuclear fragments might bear some relationship to the manner in which the mu particles disappear after the genic changes studied in Section 1, it was decided to study these fragments. Conjugation or autogamy inevitably leads to the break-up of the macronucleus in ciliate Protozoa and thus the change of genotype from $M_2$ to $m_2$ would be accompanied by macronuclear break-up.

The macronuclear fragments present at various fissions after conjugation of mate-killer animals ($m_1M_1M_2m_2$) with sensitives (513) were counted by staining with aceto-carmine. The results are shown in graphic form in Figure 3. The numbers of visible fragments per animal decreased by approximately 50% at each fission following conjugation and at the 'eight fission' stage only two in every ten animals possessed a macronuclear fragment. Thus there could be no direct association between disappearance of macronuclear fragments and loss of mu particles, which, as shown above takes place mainly between the eighth and fifteenth fissions.

(b) With starvation of ex-conjugants

Counts of the visible fragments were made as above at various stages, but in this case the ex-conjugants were starved for various periods of time. Previous work by Sonneborn (unpublished) had shown that starvation conditions reduced the number of macronuclear fragments. This finding was confirmed in the experiments reported here. With three days' starvation of ex-conjugant mate-killers and with subsequent growth in lettuce medium for three asexual fissions, only four out of 450 animals had a single macronuclear fragment.
This was strikingly different from the results with unstarved lines, where, after three fissions, eight or nine fragments per cell were observed.

Although starvation of paramecia had a remarkable effect on the behaviour of the macronuclear fragments, it has been shown in Section 2 that the manner of disappearance of the mu particles following the genic change is unaffected by such starvation.

Section 4 (a). Investigation of animals which have passed through fifteen fissions following replacement of the gene M₂ by m₂ in mate-killer cells

After the preceding experiments had been carried out it was suspected that there was another particulate body, distinct from the mu particles, present in mate-killer paramecia. This particle will be designated by the expression "metagon". More will be said of this other particle in the discussion. In order to verify the existence of metagons, however, the experiments described in the next few Sections were carried out. It was thought, for reasons to be mentioned later, that if such hypothetical particles existed only a few would be present at the later fission stages in the M₁M₁M₂M₂ clones. The animals selected for study were mate-killer animals at the 'fifteenth fission' stage following replacement of the gene M₂ by the recessive allele m₂. This genotypic change was carried out in the manner outlined on page 9.

The selection of the paramecia which had mu particles at the 'fifteenth fission' stage was not accomplished by squashing and killing the animals. Instead, paramecia at the 'fifteenth fission' stage were mated to sensitive cells and if they brought about the death of the sensitive animals they were classified as mate-killers. After thus identifying the 'fifteenth fission'
stage mate-killers they were allowed to pass through a few further fissions and the number of daughter cells with mu particles was found by squashing the cells.

It was essential to use autogamy rather than conjugation to obtain the $m_1m_2\overline{m}_1\overline{m}_2$ clones since for identification of the mate-killers at the 'fifteenth fission' stage a further conjugation was necessary. Following conjugation, ex-conjugants enter an immature period, during which conjugation could not occur for several weeks. After autogamy, however, the immature period is relatively short - at the most three days. 'Fifteenth fission' stage animals can be induced to mate after autogamy.

The actual experimental details were as follows: 478 animals at the 'fifteenth fission' stage were placed in a small drop of exhausted culture fluid along with several hundred sensitive 513 animals ($m_1m_2\overline{m}_1\overline{m}_2$) which had been fed up and then starved down in preparation for conjugation as first reported by Sonneborn (1943). In 38 cases following conjugation unilateral death was found to occur. This was concluded to be the result of matings between mate-killer and sensitive conjugants. Hence, the mate-killing phenotype was shown by 'fifteenth fission' stage animals in the absence of the genes $M_1$ and $M_2$. The proportion of cells at this stage, 38 out of 478 (7.9%) which killed unilaterally, was similar to that obtained in Section 1 when mate-killer cells following fifteen fissions after conjugation, were identified by direct observation of mu particles (7%). The ex-conjugant survivors from the 38 pairs mentioned above were allowed to undergo three asexual fissions. This yielded 38 groups of eight animals which were examined for mu particles by phase contrast microscopy. The results are shown in Table 6.
Table 6. ANALYSIS OF GROUPS OF EIGHT ANIMALS OBTAINED BY THREE FISSIONS OF 'FIFTEEN FISSION' STAGE ANIMALS (ONLY MATE-KILLERS GROWN ON)

<table>
<thead>
<tr>
<th>Mate-killers (with μ particles)</th>
<th>Sensitive (without μ particles)</th>
<th>Numbers obtained of each octet</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>
The majority of 'fifteenth fission' stage cells which brought about death of sensitive mates gave, after three further fissions, groups of eight cells consisting of 1 mate-killer and 7 sensitives. In a few cases groups of 2 mate-killers : 6 sensitives or 3 mate-killers : 5 sensitives were obtained, but none of 0 mate-killers : 8 sensitives. This result is of great significance since it shows that there is in fact a unit factor present (the netagon) which is responsible for the change from mate-killer to sensitive, following the genotypic change. This will be discussed later.

(b) The time of disappearance of mu particles following division of 'fifteenth fission' stage mate-killers

The manner in which a cell with mu particles switched to one without was studied here by phase contrast microscopy. Mate-killer 'fifteenth fission' stage animals were selected out as in the previous section, but the matings were carried out in yeast medium (see page 8). The mate-killer survivors were then removed from this medium, placed separately in drops of yeast medium and allowed to divide once more. The two daughter cells were separately squashed and examined for mu particles, some immediately following fission and some during the next inter-fission period.

It was found that immediately following the fission of the 'fifteenth fission' stage mate-killer, both cells had many mu particles, but that during the next inter-fission period one of each pair of daughter cells had fewer and fewer particles. The number of mu particles was found to diminish at about one hour after fission, and after six hours the destruction was complete. During the period when the numbers were being reduced in number, the mu particles themselves remained in
Figure 4. Method of analysis of paramecia assumed to contain a single metagon after fifteen fissions following loss of gene $M_2^*$. 
characteristic clumps (as described by Siegel, 1953), although diminishing in number, and some of them became rather indistinct.

These observations confirmed that when a 'fifteenth fission' stage cell with mu particles divided and produced two daughter cells, both daughters initially contained many mu particles but subsequently destruction of mu particles took place in one daughter cell. At the same time in the sister cell however, there must have been a multiplication of mu particles to counteract the loss of half at each fission. In Figure 4 the details of these experiments in Section 4(a) and (b) are illustrated.

Section 5 (a). Investigations of animals which have undergone eleven fissions following substitution of $M_2$ by $m_2$ in mate-killer cells

Assuming that mate-killer paramecia contain particles of the type called metagons as well as mu particles, at the 'eleventh fission' stage paramecia might be expected to contain varying numbers of metagons. In any case 'eleventh fission' stage animals would possess more metagons than in 'fifteen fission' stage animals. It was decided, therefore, to study 'eleventh fission' stage animals in a similar way to fifteenth fission cells.

Animals at the 'eleventh fission' stage after substitution of the gene $M_2$ by $m_2$ were obtained in the manner previously described ("Methods" section). They were prepared from ex-autogamous individuals (see previous section) and thus it was possible to make them conjugate with sensitive paramecia belonging to Stock 513. If the 'eleventh fission' stage paramecium brought about the death of the 513 mate it was classified as a mate-killer. After thus identifying the mate-killer 'eleventh fission'
Table 7. ANALYSIS OF GROUPS OF EIGHT ANIMALS OBTAINED BY THREE FISSIONS OF 'ELEVEN FISSION' STAGE ANIMALS (ONLY MATE-KILLERS GROWN ON)

<table>
<thead>
<tr>
<th>Mate-killers (with mu particles) : sensitives (without mu particles)</th>
<th>Numbers obtained of each octet</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 : 8</td>
<td>0</td>
</tr>
<tr>
<td>1 : 7</td>
<td>54</td>
</tr>
<tr>
<td>2 : 6</td>
<td>46</td>
</tr>
<tr>
<td>3 : 5</td>
<td>45</td>
</tr>
<tr>
<td>4 : 4</td>
<td>8</td>
</tr>
<tr>
<td>5 : 3</td>
<td>6</td>
</tr>
<tr>
<td>6 : 2</td>
<td>4</td>
</tr>
<tr>
<td>7 : 1</td>
<td>3</td>
</tr>
<tr>
<td>8 : 0</td>
<td>0</td>
</tr>
</tbody>
</table>
stage animals they were allowed to divide three times to give eight
daughter paramecia which were then squashed and examined for mu particles.

The details of these experiments were as follows: 426 animals at
the 'eleventh fission' stage were placed in a small drop of exhausted
culture fluid with several hundred sensitive Stock 513 animals \( \text{S}_{1} \text{S}_{2} \text{S}_{3} \text{S}_{4} \) which had been prepared for conjugation as described in the previous
section. In 166 cases following conjugation unilateral death was found
to occur, and these survivors were classified as mate-killers. The
proportion of cells at this state demonstrating mate-killing (39%) was
similar to that found in Section 1 when 39% of 'eleventh fission' stage
animals were classified as mate-killers after examination for mu particles
by direct observation. The ex-conjugant survivors were allowed to
divide thrice to give groups of eight animals. These groups were then
scored for mu particles by squashing each cell individually and examining
them by phase contrast. The results are shown in Table 7.

It was found that the class of 0 mate-killers : 8 sensitives was
missing as already found in the previous section dealing with the progeny
of 'fifteenth fission' mate-killers. In the present experiment groups of
1 mate-killer : 7 sensitives, 2 mate-killers : 6 sensitives, 3 mate-killers :
5 sensitives occurred in approximately equal numbers. However, groups of
and
4 mate-killers : 4 sensitives, and groups with five, six, seven
mate-killers also occurred.

These results will be interpreted in the discussion, but it suffices
here to state that they are in conformance with the view that a percentage
of 'eleventh fission' stage animals with mu particles possess a single unit
factor - the metagon - responsible for the presence of mu particles in
these cells. Other 'eleventh fission' stage paramecia must possess more
than one of these metagons.
### Table 8. TIME TAKEN BY 'ELEVEN FISSION' STAGE PARAMECIA TO KILL SENSITIVES AND ANALYSIS OF PROGENY AFTER THREE FURTHER FISSIONS

<table>
<thead>
<tr>
<th>Time of killing (in hours)</th>
<th>Proportion of cells with : without mu particles three fissions later</th>
<th>Number of 'eleven fission cells in this class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 : 1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6 : 2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 : 3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4 : 4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>3 : 5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2 : 6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1 : 7</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>7 : 1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6 : 2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 : 3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4 : 4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3 : 5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2 : 6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1 : 7</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>7 : 1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6 : 2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 : 3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4 : 4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3 : 5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2 : 6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1 : 7</td>
<td>2</td>
</tr>
<tr>
<td>24</td>
<td>7 : 1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6 : 2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 : 3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4 : 4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3 : 5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2 : 6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>1 : 7</td>
<td>8</td>
</tr>
<tr>
<td>48</td>
<td>7 : 1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6 : 2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5 : 3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4 : 4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3 : 5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2 : 6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1 : 7</td>
<td>7</td>
</tr>
</tbody>
</table>
(b) **The speed of mate-killing as demonstrated by 'eleventh fission' stage animals**

The time taken by 'eleventh fission' stage cells to kill sensitive Stock 513 animals was noted (see (a) above). The survivors were considered to be mate-killers. Measurements were made of the time from the separation of the conjugants until only one of the ex-conjugants could still be seen. Each mate-killer was then allowed to pass through three asexual fissions as in the experiment already mentioned above. The results of this time experiment are shown in Table 8.

It will be seen that there is a relationship between speed of killing and the number of mate-killers three fissions later. If an animal kills in six hours then groups of eight animals with seven mate-killers were found three fissions later, whereas if an animal kills in 48 hours then only groups of one, two or three cells with mu particles were found (Table 8). The significance of this observation will be discussed later.

**Section 6.** **The effect of cytoplasmic exchange on the numbers of mate-killers at the later fissions following conjugation between paramecia at the 'seventh and fifteenth fission' stages**

Although cytoplasmic exchange does not normally occur at conjugation it is possible by special treatment to bring about such exchange, and this was done with the aim of studying the localisation within the cell of the hypothetical units called metagons. The animals used in these experiments were at the 'seventh fission' stage after gene replacement (all animals were of genotype $m_1m_1m_2m_2$). They were mated to animals at the 'fifteenth fission' stage (same genotype). The former animals possessed mu particles, and were therefore mate-killers. The 'fifteenth fission' stage animals as shown in Section 3 consisted of 7% mate-killers with mu particles, while 93% of them were sensitives and lacked mu particles.
In order to identify the exact percentages of mu particles containing cells from each ex-conjugant, the mates were marked with the immobilisation antigens (see Beale, 1954). 'Seventh fission' stage cells were prepared in the manner outlined on page 9, but in addition they were selected out in clones shown to bear the antigenic marker 90G, while the 'fifteenth fission' stage cells were prepared from clones marked with the antigenic type 60G. The daughter cells from each ex-conjugant could then be traced to their sources, i.e. from the 'seventh fission' stage mate of the 'fifteenth fission' stage mate. At the same time it was possible five fissions after the mating to ascertain that nuclear exchange had in fact taken place, since both lines of daughter cells would, by this stage, be expressing both antigenic types (see Beale, 1954 for details).

Cytoplasmic exchange was induced by treating the 'seventh fission' stage and 'fifteenth fission' stage conjugants with dilute anti-60G and anti-90G sera at a time of one hour before the normal end of conjugation, i.e. five hours after the pairs had began to conjugate. The pairs were removed from the anti-serum after a further six hours and placed separately in bacterised medium. In about 10% of the pairs broad cytoplasmic bridges had formed after about four hours in the serum, and remained for another six hours before final separation of the conjugants in normal medium. These pairs were then used for the experiments to be reported here. A control experiment was carried out without cytoplasmic exchange. After separation of the conjugants, the ex-conjugants were allowed to divide three times, giving groups of eight animals of which seven were examined for mu particles and the eighth allowed to pass through three further fissions, when the resulting cells were examined for mu particles. The
Figure 5. Results of experiments involving conjugations with and without cytoplasmic exchange.
results are shown in Figure 5.

In the control experiment only pairs showing bilateral survival were carried on and their progeny examined for mu particles. These matings were interpreted as occurring between 'seventh fission' stage animals and 'fifteenth fission' stage animals with mu particles. Another 81 pairs gave unilateral death of the 60G ex-conjugant derived from 'fifteenth fission' stage mates lacking mu particles, but the 81 survivors were discarded. In the experimental series with cytoplasmic exchange however, there was no rejection of one group of matings. Six successful conjugants were obtained, accompanied by cytoplasmic exchange. In all six cases, both ex-conjugants survived. Since only 8% of 'fifteenth fission' stage cells are mate-killers, by chance it is likely that in these experiments most or all of the six 'fifteenth fission' stage conjugants were sensitives. In the control, however, the 'fifteenth fission' stage cells, selected by the technique above, always possessed mu particles.

The results in Figure 5 show clearly that in the experimental series mu particles passed from one mate to the other via the cytoplasmic bridge. All eight daughter cells derived by asexual fission from the 'fifteenth fission' stage mate which had received cytoplasm from the 'seventh fission' stage animal, contained mu particles. This was found to be the case for all six clones by direct examination of seven animals under phase contrast, and in the case of the eighth animal by inference from the scoring of the later progeny. In the control experiment 32 out of 80 (40%) paramecia had mu particles after six fissions of the ex-conjugants derived from the 'seventh fission' stage conjugant and 6% from the 'fifteenth fission' stage conjugant. When cytoplasmic exchange occurred in the experimental series, however, the respective percentages were 71% and 73%.
Table 9. ANALYSIS OF GROUPS OF EIGHT CELLS FOLLOWING CONJUGATION OF ELEVEN FISSION STAGE ANIMALS WITH STOCK 513 SENSITIVES AND INDUCED CYTOPLASMIC EXCHANGE

<table>
<thead>
<tr>
<th>Progeny&lt;sup&gt;x&lt;/sup&gt; of eleven fission stage conjugant</th>
<th>Progeny&lt;sup&gt;x&lt;/sup&gt; of Stock 513 (sensitive) conjugant</th>
<th>Numbers found</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 : 8</td>
<td>0 : 8</td>
<td>22</td>
</tr>
<tr>
<td>1 : 7</td>
<td>0 : 8</td>
<td>11</td>
</tr>
<tr>
<td>0 : 8</td>
<td>1 : 7</td>
<td>2</td>
</tr>
<tr>
<td>1 : 7</td>
<td>1 : 7</td>
<td>8</td>
</tr>
<tr>
<td>0 : 8</td>
<td>2 : 6</td>
<td>1</td>
</tr>
<tr>
<td>1 : 7</td>
<td>2 : 6</td>
<td>4</td>
</tr>
<tr>
<td>2 : 6</td>
<td>1 : 7</td>
<td>3</td>
</tr>
<tr>
<td>0 : 8</td>
<td>3 : 5</td>
<td>1</td>
</tr>
<tr>
<td>1 : 7</td>
<td>Dead</td>
<td>1</td>
</tr>
<tr>
<td>2 : 6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>x</sup> Antigen markers 60G and 90G were used for identification of ex-conjugants.
Further experiments utilising cytoplasmic exchange were carried out but involving conjugation between 'eleventh fission' stage animals and Stock 513 sensitives. In accordance with our results in Section 5(a) some 39% of the 'eleventh fission' stage mates were expected to be mate-killers. The results are shown in Table 9. It was found that in fifteen pairs, one of the ex-conjugants yielded eight animals without mu particles, while the other yielded one or more paramecia with mu particles. These particular results show clearly that:

(a) with cytoplasmic exchange between mate-killer and sensitive conjugants, the latter are not killed but can give rise to eight sensitive paramecia. Siegel (1953) had found in Syngen 8 that cytoplasmic exchange could in many cases lead to death of the sensitive conjugant in a mate-killer - sensitive cross. However, in some cases bilateral survival was observed. In our experiments involving cytoplasmic exchange, however (see later), only few cases (6%) result in unilateral death.

(b) mu particles and metagons can pass to Stock 513 mates from 'eleventh fission' stage conjugants and as a consequence the original mate-killer cell can become a sensitive, and vice-versa.

In twenty two pairs, however, all the progeny scored were sensitives. This is in accordance with the results in Section 5(a) where 61% of the 'eleventh fission' stage paramecia were found to be sensitives.

Section 7. Further experiments with cytoplasmic exchange

In the previous section it was shown that, in crosses involving 'seventh fission' stage animals and 'fifteenth fission' stage animals accompanied by cytoplasmic exchange, the number of paramecia with mu particles (100%) following three fissions after separation of the 'seventh fission' stage conjugants, i.e. at the 'tenth fission' stage, was in excess of the number obtained - 68% - without cytoplasmic exchange at this 'tenth fission' stage (Section 1, Table 1).
Table 10. EFFECT OF CYTOPLASMIC EXCHANGE ON THE NUMBER OF CELLS WITH MU PARTICLES FOLLOWING CONJUGATION OF THIRD FISSION STAGE ANIMALS WITH STOCK 513 ANIMALS

<table>
<thead>
<tr>
<th>Percentage of cells with mu particles after fifteen fissions</th>
<th>Percentage of cells with mu particles after eleven fissions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progeny&lt;sup&gt;x&lt;/sup&gt; of third fission stage animal</td>
<td>Progeny&lt;sup&gt;x&lt;/sup&gt; of Stock 513 (sensitive animal)</td>
</tr>
<tr>
<td>5% (8%)</td>
<td>9% dies</td>
</tr>
</tbody>
</table>

The figures in brackets were obtained from a control experiment without cytoplasmic exchange, when one of the conjugants dies because of 'mate-killing'.

<sup>x</sup> Antigen markers 60G and 90G were used for identification of ex-conjugants.
To test the effect of cytoplasmic exchange on the proportion of cells finally containing mu particles, cells at the 'third fission' stage after replacement of the gene $M_2$ by $m_2$ were mated to sensitive Stock 513 animals without mu particles. Cytoplasmic exchange was induced between the pairs. 'Third fission' stage animals, of course, possessed mu particles. By allowing the ex-conjugants to divide asexually until the 'eleventh fission' stage and 'fifteenth fission' stage and by scoring the progeny cells it was expected that the effects of cytoplasmic exchange would be evident.

A control experiment was carried out with conjugation between 'third fission' stage animals and sensitive 513 animals, but without cytoplasmic exchange. After separation the ex-conjugants were each allowed to divide six times and 32 of the resultant 64 animals picked out and allowed to divide once in the manner already outlined on page of the "Methods" section. At the 'eleventh fission' stage 50 of the 64 animals were examined for presence or absence of mu particles. In some cases ex-conjugants were taken to the 'fifteenth fission' stage in the same way and animals were scored. The control series was carried out in the same way. The results are shown in Table 10.

From the results it is clear that in the series of experiments with cytoplasmic exchange, there are many more paramecia with mu particles at the 'eleventh fission' scoring stage than in the accompanying control series. By contrast, however, similar percentages of mate-killer paramecia at the 'fifteenth fission' stage were found in both the control and experimental series.

Section 8 (a). Studies on the re-introduction of the gene $M_2$ into 'fifteenth fission' stage paramecia of genotype $m_1m_2m_2$.

So far this thesis has been concerned with the effect of substitution
Figure 6. Effect of re-introducing the gene $m_2$ into $m_1 m_1 m_2 m_2$ animals assumed to contain one metagon and $m_2$ particles.
Table 11. **EFFECT OF RE-INTRODUCTION OF GENE M$_2$ INTO FIFTEEN STAGE ANIMALS (MATE-KILLERS)**

<table>
<thead>
<tr>
<th>Number of fissions after re-introduction of gene M$_2$</th>
<th>Proportion of animals with : without mu particles</th>
<th>Number of clones found</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2 : 2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>4 : 4</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>8 : 8</td>
<td>5</td>
</tr>
</tbody>
</table>

* No other ratios were found
of the determining gene $M_2$ by the recessive $m_2$ on the growth and maintenance of $mu$ particles in the cytoplasm of paramecia. In this section the effect of re-introduction of the gene $M_2$ into animals of genotype $m_1m_1m_2m_2$ will be investigated. It was necessary to carry out this experiment with paramecia possessing $mu$ particles since once the particles are lost they cannot be re-synthesised merely by introduction of the dominant gene.

Conjugations were brought about as shown in Figure 6 between animals at the 'fifteenth fission' stage (genotype $m_1m_1m_2m_2$) and sensitive animals without $mu$ particles derived from Tester 7 ($m_1m_1M_2M_2$) - (see "Materials" section). Where unilateral death of the sensitive mate occurred the 'fifteenth fission' stage animal was considered to be a mate-killer. This occurred in about 8% of the cases (see Table 1). Accompanying each conjugation, however, there is a nuclear exchange preceded in each mate by meiosis (Sonneborn, 1950). The result of the above cross would be then to make the surviving 'fifteenth fission' mate a mate-killer cell of genotype $m_1m_1M_2m_2$. Each ex-conjugant was then allowed to undergo three fissions and the progeny scored for $mu$ particles. The results are shown in Table 11.

These results show that re-introduction of the gene $M_2$ resulted in formation of groups of eight paramecia comprising four mate-killers and four sensitives. It is known from the experiments reported in Section 4(a) that 'fifteenth fission' mate-killers yield groups of one mate-killer and seven sensitives in most cases, and occasionally groups of two mate-killers and six sensitives or even three mate-killers and five sensitives. At no time were groups consisting of four mate-killers and four sensitives recovered. The results in this section, however, were interpreted to mean
Table 12. EFFECT OF RE-INTRODUCTION OF GENE $M_2$ INTO ELEVEN FISSION STAGE ANIMALS (MATE-KILLERS)

<table>
<thead>
<tr>
<th>Number of fissions after re-introduction of gene $M_2$</th>
<th>Proportion of animals with : without mu particles</th>
<th>Number of clones found</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>$4 : 4$</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>$8 : 8$</td>
<td>6</td>
</tr>
<tr>
<td>16 : 0</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
that the introduction of the gene $M_2$ was followed by the appearance of metagons in daughter cells essential for the growth of mu particles. The metagons appeared some time during the 'second fission' stage following conjugation, but not during the 'first fission' stage. This is the only explanation which fits the observation of 4:4 groups of mate-killers and sensitives, since if the metagons appeared during the 'first fission' stage all eight animals scored would be mate-killers.

(b) Studies on the re-introduction of the gene $M_2$ into 'eleventh fission' stage paramecia of genotype $m_1m_1m_2m_2$

A further series of experiments was carried out with 'eleventh fission' stage mate-killer paramecia (selected as in Section 5) and sensitive animals ($m_1m_1m_2m_2$) in the same manner as described with 'fifteenth fission' stage cells. As shown in the results in Table 12, groups of 4 mate-killers : 4 sensitives were derived from the survivor after three fissions. This is interpreted in the same way as in the previous section since the 'eleventh fission' stage animals have mu particles. There is a one fission delay before the gene $M_2$ effects the appearance of metagons. This explanation would also explain the 8 mate-killers and 8 sensitives found after one more fission (i.e. four fissions in all). The one exception to this is shown in Table 12 where sixteen mate-killers were found, but this class will be explained in the discussion.
RESULTS

PART II

Assuming that "metagons" exist, they could be composed of ribonucleic acid (RNA), deoxyribonucleic (DNA) or protein. In an attempt to determine the chemical nature of the metagon, mate-killer paramecia were treated with ribonuclease and 8-azaguanine, two substances which have been shown to affect ribonucleic acid in characteristic ways.

Section 1. Effects of Ribonuclease on Stock 540 mate-killers

(a) Without starvation following treatment

Stock 540 mate-killers of genotype \( M_1 \bar{M}_2 M_2 M_2 \) with mu particles were treated with 1 ml. RNAase solution (0.5 mg/ml) for 12 hours at 20\(^\circ\)C. The survivors (10%) were then placed in normal culture medium for various times at 25\(^\circ\)C and scored for the presence or absence of mu particles. There was a long delay before the first fission at 25\(^\circ\)C - usually about 48 hours - in place of the normal 8 hours - due to the action of the ribonuclease. After the first fission, but not before, there was found to be a remarkable reduction in numbers of mu particles. Before the second fission was completed mu particles could not be seen in any of the daughter cells. The second fission after treatment took place about 8 hours after the first fission (i.e. in all, 56 hours after treatment) at 25\(^\circ\)C.

(b) With starvation following treatment

Some Stock 540 paramecia after treatment with the enzyme (as in (a) above) were placed in non-nutrient culture fluid and were maintained there, without dividing, for 96 hours. They were then placed in normal lettuce culture fluid and underwent one fission 24 hours later at 25\(^\circ\)C. Samples of these animals were taken at various times, e.g. before and after the first fission,
and were scored for presence or absence of mu particles. Animals were also scored at various periods between the second and third fissions. Once more it was found that the mu particles began to disappear after the first fission and not before.

The results in Sections (a) and (b) showed that however long the delay before the first fission, whether 48 or 120 hours, large numbers of mu particles continued to be maintained in the cytoplasm. After the first fission, however, in both cases, there was a remarkable reduction in numbers of mu particles and before the second fission was completed they had completely disappeared from all paramecia, which were converted to sensitives. At later stages after treatment, e.g. after 8 and 15 fissions, the mu particles were never seen to re-appear and all the cells remained permanently as sensitives.

Section 2. **Effect of exposure to ribonuclease on mate-killer paramecia in the absence of the gene \( M_2 \)**

About sixty 'seventh fission' stage mate-killers (genotype \( m_1m_1m_2m_2 \)) were treated with 1 ml. ribonuclease solution (conc. .5 mg/ml) for 12 hours at room temperature (20°C). A large proportion (90%) of the animals died and all the surviving animals (10%) were slowed down in their swimming movements. After recovery, which took about 48 hours, the surviving animals were mated before dividing to untreated 'eleventh fission' stage animals (also of genotype \( m_1m_1m_2m_2 \)). Previous work (Section 5) had shown that the latter animals comprised the following percentages in terms of mate-killers and sensitives, 61% sensitives, 39% mate-killers.

After the mating the animals were allowed to separate and to undergo three asexual fissions. The animals at this stage were then examined for mu particles. Control matings were also made between 'seventh fission' stage animals which had not previously been treated with ribonuclease and 'eleventh fission' stage animals (likewise untreated).
Figure 7. Ribonuclease treatment of "seventh fission" stage animals (see text). On left, treated series; on right, untreated control series. (Antigen markers 60G and 90G were used for identification of ex-conjugants).
Since the object of these experiments was to determine the effect of ribonuclease on the treated 'seventh fission' stage animals and since the mu particles had been shown (Section 1) not to persist in treated animals, it was necessary to re-introduce the mu particles by cytoplasmic exchange between the treated 'seventh fission' stage animals and untreated 'eleventh fission' stage animals. When such exchange involved an 'eleventh fission' animal containing mu particles by study of the progeny it would be possible to see if there were more progeny with mu particles obtained from the 'eleventh fission' ex-conjugant than would normally be obtained from 'eleventh fission' stage animals. (Section 5, Part.1, Table 7). It could then be concluded whether or not the treated 'seventh fission' stage animals had contributed any metagons allowing mu particles to be maintained in the daughter cells. The results of these experiments are shown in Figure 7. They showed that the number of daughter cells found to be mate-killers was compatible with the assumption that in eight clones all contained metagons; the killer animals A derived from the untreated 'eleventh fission' stage ex-conjugant and none from the RNA-ase-treated 'seventh fission' conjugants. The thirteen clones in which all the daughter cells were sensitives were assumed to be derived from pairs each consisting of a 'seventh fission' stage mate-killer and 'eleventh fission' stage sensitive animal. The 'seventh fission' stage animal is assumed to have had its mu particles eliminated by exposure to ribonuclease and the 'eleventh fission' stage mate was a sensitive animal in accordance with Table 7, where it was shown that 61% of 'eleventh fission' stage animals were sensitives. In the control experiment nearly all the animals derived by asexual fission from each mate and scored after three fissions were found to be mate-killers, showing that the 'seventh fission' stage ex-conjugant had contributed some
Table 13. **EFFECT OF CONJUGATION BETWEEN 'SEVENTH FISSION' PARAMECIA PREVIOUSLY TREATED WITH RIBONUCLEASE AND UNTREATED PARAMECIA OF STOCK 540 (SENSITIVE). PRESENCE OR ABSENCE OF MU PARTICLES SCORED FOLLOWING THREE FISSIONS AFTER SEPARATION OF CONJUGANTS**

<table>
<thead>
<tr>
<th>Progeny from 'seventh fission' mate</th>
<th>Progeny from Stock 540 mate</th>
<th>Number of pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mate-killers</td>
<td>Sensitive</td>
<td>Mate-killers</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>
'factor' essential for the maintenance of the mu particles in the daughter progeny. This 'factor' already termed the "metagon" was eliminated by ribonuclease in the experimental series. The metagons must also have been able to pass into the 'eleventh fission' stage mate by way of the cytoplasmic bridge in the control series, since there were found to be a larger number of paramecia with mu particles from the 'eleventh fission' ex-conjugant than were normally obtained without cytoplasmic exchange.

Section 3. Experiments to show that the ribonuclease effect is not directly on the mu particles

About sixty 'seventh fission' stage animals (genotype \( m_1m_1m_2m_2 \)) were treated with 1 ml. of ribonuclease solution (.5 mg/1 ml) at 20°C for 12 hours. The survivors (10%) recovered after 24 hours and during the next 24 hours at 25°C before dividing they were crossed to Stock 540 sensitives, i.e., those of genotype \( M_1M_1M_2M_2 \) but lacking mu particles. Cytoplasm was exchanged between the mates and after separation of the conjugants, the ex-conjugants underwent three fissions. The resultant animals were then scored. Four pairs of ex-conjugant clones were obtained. The results are shown in Table 13. The results showed that some mu particles must have remained alive in the 'seventh fission' stage animals after ribonuclease treatment, since the Stock 540 mates did not possess any. As a result of cytoplasmic exchange both ex-conjugants received the viable mu particles.

Section 4 (a). Effect of the gene \( M_2 \) on mate-killer paramecia following exposure to ribonuclease

Animals of the genic constitution \( m_1m_1M_2M_2 \) and containing mu particles were derived from Tester 7 by crossing to Stock 513. These animals were then treated with 1 ml. RNAase solution for twelve hours (.5 mg/1 ml.) which
would be expected to remove all the metagons and indirectly the mu particles as described in Section 2.

It was desired to find out at what time, if any, regeneration of metagons would take place due to the action of the gene \( M_2 \). The analysis was complicated by the fact that the ribonuclease removed not only the metagons (Sections 1 and 2), but also indirectly the mu particles, from the progeny of the treated animals. It was thus necessary to introduce into the cytoplasm of the latter some mu particles from an untreated 'eleventh fission' stage animal by conjugation and induced cytoplasmic exchange. Whether or not the introduced mu particles could be maintained in the progeny could then be determined. In addition it was necessary to select ex-conjugants from the latter crosses not containing the gene \( M_2 \) so that the situation regarding any possible metagon re-synthesis could be 'frozen' at the time of conjugation.

Following treatment with ribonuclease, cells of genotype \( m_1m_1M_2m_2 \) were allowed to divide once, twice, four times or eight times. After the particular fission under study (i.e., 1. 2, 4 or 8) conjugation of the cells was brought about with 'eleventh fission' stage cells \( m_1m_1m_2m_2 \) and cytoplasmic exchange induced. If the gene \( M_2 \) was present after mating then the re-synthesis of metagons might occur and obscure any effect the same \( M_2 \) gene had had between ribonuclease treatment and mating. The ideal situation for the experiments to be reported here would be where the gene \( M_2 \) would only function between treatment and mating and not after conjugation. After conjugation isolation of the clones lacking the dominant gene \( M_2 \) was therefore essential.

The method of identification of the desired \( m_1m_1m_2m_2 \) clones was carried
To face page 34.

Table 14. TIME OF RE-SYNTHESIS OF METAGONS IN ANIMALS OF GENOTYPE m<sub>1</sub>m<sub>2</sub>M<sub>1m<sub>2</sub>M<sub>1</sub>-<sub>1</sub>-<sub>1</sub> FOLLOWING TREATMENT WITH RIBONUCLEASE (see TEXT)

<table>
<thead>
<tr>
<th>Stage after treatment at which mating to '11th fission' animal occurs</th>
<th>No. of pairs with cytoplasmic exchange</th>
<th>Bilateral death</th>
<th>Unilateral death</th>
<th>Analysis of groups of eight animals derived (a) from the treated animal</th>
<th>(b) from the untreated animal</th>
<th>Number of clones with the indicated proportions of animals with mu particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>With mu particles</td>
<td>Without mu particles</td>
<td>With mu particles</td>
</tr>
<tr>
<td>1 fission</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>2 fission</td>
<td>12</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>7</td>
<td>x</td>
</tr>
<tr>
<td>4 fission</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>8 fission</td>
<td>56</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

x In these cases the eighth daughter animal was allowed to pass through seven further fissions (thus arriving at the 'eleventh fission' stage) and the progeny scored for mu particles. The percentage of mate-killer cells with mu particles was then found to be 37%. 
out in the following way. After each pair of ex-conjugants had separated they were allowed to divide once. The two cells from the 'eleventh fission' stage ex-conjugant were placed in a drop of the non-nutrient culture fluid. One of the daughter cells from the treated mate ('held-back' animal) was placed in a similar drop while its partner was allowed to divide in bacterised medium for about eight fissions and the resultant cells were scored for mu particles. In some cases seven further fissions were necessary for certain identification of the \( m_{1m_1m_2m_2} \) clones. In this way it was possible to differentiate between the two genotypes \( m_{1m_1m_2m_2} \) and \( m_{1m_1m_2m_2} \). Having in this way identified the desired \( m_{1m_1m_2m_2} \) progeny the 'held-back' animals were placed in nutrient medium and allowed to undergo three fissions. The groups of eight animals thus obtained were then examined for mu particles. The results are shown in Table 14.

It will be seen that when the mating was carried out with animals following two, four and eight fissions after enzyme treatment, a re-synthesis of metagonos must have occurred in the progeny of this treated cell. This conclusion was drawn since the animals scored were all mate-killers and the 'eleventh fission' stage mate-killer would, besides donating the mu particles, only give rise to a few mate-killers (see page 21). As shown in the results (Table 14), one of the eight daughter cells derived from a cross involving cells two fissions after ribonuclease treatment was allowed to undergo seven further fissions, i.e. to reach the 'eleventh fission' stage following mating. These animals were then classified and the percentage of mate-killers found to be 37%. As a control we shall consider the case where the cell was not treated with RNAase and where the gene \( M_2 \) was replaced by \( m_2 \) as in our earlier experiments. When cells were then allowed to pass through eleven fissions and the progeny scored for the
presence of mu particles, 39% of the animals had mu particles at this stage. The result was quite different when experiments were carried out with cells which had undergone only one fission after enzyme treatment. These cells were not found to possess metagons - the percentage of mate-killers after classification was in accordance with the view that the mate-killers obtained had arisen from the 'eleventh fission' mate.

The general conclusion from these results was that after removal of the mu particles by treatment with ribonuclease in the presence of the gene M, metagons re-appeared at a stage just following the second fission after enzyme treatment.

(b) **Cytological effect of exposure to ribonuclease of mate-killers containing the gene M**

Stock 540 mate-killer animals (H1H1H2H2) were treated with ribonuclease (5 mg/ml) for six hours. The animals were then examined, after fixation in osmic acid, in the electron microscope. The effect on the cytoplasm is shown in Plate Six. There is a drastic reduction in the number of fine granules and it has also been shown that pyronin staining ability disappeared after ribonuclease treatment. Some of the surviving animals after treatment were allowed to divide once (48 hours at 25°C) and then fixed and examined by the electron microscope at this the 'second fission' stage. The result of this procedure is shown in Plate Seven.

At the 'second fission' stage it will be seen that the fine granules re-appear in the cytoplasm of mate-killer paramecia and also incidentally so does the ability to be stained with pyronin staining.

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This part of the work was done in collaboration with Dr. A. Jurand, to whom I give thanks.
Table 16. TREATMENT OF SEVENTH FISSION HATE-KILLERS WITH DIFFERENT CONCENTRATIONS OF RIBONUCLEASE FOR VARIOUS EXPOSURE TIMES AND THE EFFECTS ON THE NUMBER OF HATE-KILLERS, FOUR FISSIONS LATER

<table>
<thead>
<tr>
<th>Conc. of RNAase</th>
<th>Time of treatment (hours)</th>
<th>Number animals treated</th>
<th>Percentage Survival</th>
<th>Percentage hate-killers four fissions later (eleventh fission stage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39(^x)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>60</td>
<td>95</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50</td>
<td>95</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>60</td>
<td>85</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>50</td>
<td>80</td>
<td>25</td>
</tr>
<tr>
<td>0.125 mg/1 ml.</td>
<td>1</td>
<td>50</td>
<td>98</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>60</td>
<td>95</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>50</td>
<td>74</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>60</td>
<td>94</td>
<td>18</td>
</tr>
<tr>
<td>0.25 mg/1 ml.</td>
<td>8</td>
<td>70</td>
<td>90</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>65</td>
<td>83</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>60</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>0.5 mg/1 ml.</td>
<td>1</td>
<td>60</td>
<td>80</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50</td>
<td>25</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>60</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>50</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

\(^x\) Data from Section 1, Table 1.
Section 5. The effects on mate-killers of varying the concentration and time of exposure to ribonuclease

(a) Variation of exposure time with a constant concentration of enzyme

The effect of varying times of exposure to RNAase was studied by taking a series of 'seventh fission' stage mate-killers and treating samples for varying periods of time between 1-12 hours with RNAase (.25 mg/ml). The experiment was repeated using a concentration of .5 mg RNAase/1 ml for one, two, four and six hours and again with .125 mg RNAase/1 ml for the same intervals of time. After each treatment four of the survivors were removed from the RNAase solution and allowed to recover and to divide four times in bacterised medium (the first fission taking two days). The resultant progeny were then examined for mu particles at this time, i.e., at the 'eleventh fission' stage after substitution of the gene $M_2$ by $M_2$. The results are shown in Table 15. These show that the percentage of mate-killers at the 'eleventh fission' stage diminished with an increasing time of exposure to a given enzyme concentration. Without treatment, the percentage of mate-killers at this stage was 39% (see Table 1). It is interesting to point out that even after one hour's exposure to the enzyme there would appear to be a slight though detectable effect. It remains possible that the entry of the ribonuclease sufficient for a subsequent effect took place in an even smaller time interval than one hour.

(b) Variation of enzyme concentration with a constant time of exposure (6 hours)

Four groups of fifty 'seventh fission' mate-killers were exposed for six hours to different concentrations of ribonuclease (.125, .375, .5, .625 mg/ml)
Table 15. Treatment of 'seventh fission' mate-killers with different concentrations of ribonuclease for a constant exposure time (6 hours) and the effect on the number of mate-killers four fissions later ('eleventh fission' stage)

<table>
<thead>
<tr>
<th>Concentration of RNase</th>
<th>Number animals treated</th>
<th>Percentage Survival</th>
<th>Percentage Mate-killers at 'eleventh fission' stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>39&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>.125</td>
<td>50</td>
<td>75</td>
<td>27</td>
</tr>
<tr>
<td>.25</td>
<td>40</td>
<td>70</td>
<td>17</td>
</tr>
<tr>
<td>.375</td>
<td>50</td>
<td>44</td>
<td>14</td>
</tr>
<tr>
<td>.5</td>
<td>55</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

<sup>x</sup> = from Table 1
Table 17. EFFECT OF TREATMENT OF HATE-KILLER PARAMECIA WITH 8-AZAGUANINE

<table>
<thead>
<tr>
<th>Concentration of 8-azaguanine solution (mg/ml)</th>
<th>Number of cells examined</th>
<th>Percentage animals with mu particles after six hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>.05</td>
<td>25</td>
<td>0%</td>
</tr>
<tr>
<td>.025</td>
<td>20</td>
<td>0%</td>
</tr>
<tr>
<td>.0125</td>
<td>25</td>
<td>60%</td>
</tr>
</tbody>
</table>
The survivors were transferred to lettuce culture fluid and after recovery were allowed to divide four times. The sixteen animals so obtained from each treated animal were then classified as before. The results are shown in Table 16. It is clear that with an increasing concentration of ribonuclease there were decreasing proportions of animals containing mu particles at the 'eleventh fission' stage.

Section 6. Effects of varying concentrations of 8-azaguanine on Stock 540 mate-killers with a constant exposure time

Stock 540 mate-killers (N.M.M.M.) with mu particles were treated in a series of experiments with different concentrations of 8-azaguanine solution, viz., .05 mg/1 ml., .025 mg/1 ml. and .0125 mg/1 ml. Each series of animals was exposed to the analogue for six hours. The survivors in each case were taken from the 8-azaguanine solution, squashed and examined for presence or absence of mu particles under phase contrast. The results are shown in Table 17.

After treatment with 8-azaguanine the mu particles were found to disappear from the cytoplasm of the cells. In this case, however, the mu particles had disappeared by six hours after exposure to the analogue (i.e., before the first fission). This contrasts with the results in Section 1, where, after ribonuclease treatment, the mu particles remain present for long periods of time and only disappear after one fission.

Section 7. Experiments to show that the effect of 8-azaguanine is not on the metagons but directly on mu particles

About sixty 'seventh fission' stage mate-killer animals (genotype m_1m_2m_3m_4) containing mu particles were treated with 1 ml. of 8-azaguanine solution (.125 mg/1 ml) at 20°C for six hours. All the animals
Figure 8. Analysis of groups of eight animals obtained following conjugation of "seventh fission" stage animals (previously treated with 8-azaguanine) and "eleventh fission" stage animals with induced cytoplasmic exchange.
were slowed down in their swimming movements and 75% of them died. The surviving animals were placed in lettuce medium, recovered after 48 hours and within the next 48 hours were crossed before dividing, to 'eleventh fission' animals (genotype $m_1^2m_2^2m_3^2$). Cytoplasm was exchanged between the mates in only three pairs and after separation of the conjugants the ex-conjugants were allowed to undergo three asexual fissions in normal lettuce medium, the first fission taking eight hours. The eight daughter cells from each ex-conjugant were examined for presence of mu particles. The results are shown in Figure 3.

Since the mu particles have been shown in the previous section to be removed by 8-azaguanine at the concentration used here, the four groups of eight cells all containing mu particles must have received mu particles from amongst those originally present at mating in the untreated 'eleventh fission' stage cell. It has already been pointed out that of the 'eleventh fission' cells 39% are mate-killers. The proportion of mate-killers found at the third fission after conjugation following 8-azaguanine treatment (see Fig. 3) is too large to be explicable by derivation of mu particles and metagons from the 'eleventh fission' stage cell. It is therefore concluded that the treated 'seventh fission' stage cell, although losing its mu particles, maintained the metagons which were originally present before exposure to 8-azaguanine. It is assumed that the two groups of eight cells which were all sensitives, were derived from an original cross of a treated 'seventh fission' stage cell and an 'eleventh fission' stage sensitive. Neither of these conjugants would be expected to possess mu particles.

Hence, treatment of mate-killer cells with 8-azaguanine resulted in direct elimination of mu particles but the metagons were apparently not
affected. This was contrary to the situation with ribonuclease.

Section 8. Inhibitory effect of 8-azaguanine at the second fission following exposure to ribonuclease

It was now desired to determine whether 8-azaguanine, though it did not apparently interfere with the pre-existing metagons nevertheless was capable of inhibiting the re-appearance of these metagons. This was done by first removing the metagons from a sample of paramecia containing the gene $M_2$ with ribonuclease, and subsequently treating the animals with 8-azaguanine at a time - 1 - 2 fissions after RNAase treatment - when regeneration of the metagons was expected to take place as found by previous experiments (Section 4). Following the 8-azaguanine treatment the animals were tested by appropriate procedures to determine if metagons were present. The details of this experiment are as follows: Animals of the genotype $m_1^{-}m_2^{-}M_2^{-}$ (Tester 7), possessing mu particles were exposed to RNAase solution for twelve hours (.5 mg/ml). As reported in Section 2 above, it is known that this concentration of enzyme was sufficient to eliminate metagons and the mu particles from the treated cells. The survivors were taken from the RNAase solution, placed in lettuce medium and underwent one asexual fission within 48 hours. The daughter cells were then taken from the lettuce medium and exposed to a solution of 8-azaguanine (.05 mg/ml) at 20°C. They were left in this solution for twelve hours and then returned to lettuce medium. They underwent a further fission in about three days at 25°C. After this fission - the second after ribonuclease treatment - the paramecia were mated to 'eleventh fission' cells ($m_1^{-}m_2^{-}m_2^{-}$) and cytoplasmic exchange induced between the pairs. The ex-conjugants (of genotype $m_1^{-}m_2^{-}M_2^{-}$) were permitted to divide three times to give eight daughter cells which werestarved for twenty four hours and scored for
Figure 9. Inhibitory effect of 8-azaguanine on re-synthesis of metagons at second fission following ribonuclease treatment of animals containing $M_2$. 

- = mate-killer with mu particles
- = sensitive without mu particles
presence or absence of mu particles. A control experiment was carried out with cells treated with ribonuclease, passed through two fissions without exposure to 8-azaguanine with subsequent mating to 'eleventh fission' stage cells and with cytoplasmic exchange as before. The results of these experiments are shown in Figure 9.

The results showed that although as shown in Section 4 the re-synthesis of metagonfs took place following the second fission after ribonuclease treatment in the presence of the gene $M_2$, in the presence of 8-azaguanine this synthesis was inhibited. This conclusion was inferred from the fact that in the control experiment in the groups of eight containing any paramecia with mu particles, all or nearly all the animals had them. In fact the percentage of mate-killers was 40%. When all the cells in a group of eight were sensitives this was interpreted to be the result of a cross of a treated cell with an 'eleventh fission' sensitive (for fuller explanation see Section 2).

When the cells were exposed to 8-azaguanine (following ribonuclease treatment), however, there were found to be fewer mate-killers at the time of scoring (see Figure 9). Once more groups consisting entirely of sensitives were obtained. Occasionally groups in which four of the eight were sensitives and four were mate-killers were found. These were interpreted to be the result of the action of the gene $M_2$, two fissions after mating and will be explained at greater length in the discussion. It will also be seen that in this experiment with 8-azaguanine groups of eight cells which were all mate-killers were found. These will also be interpreted in the discussion.

In spite of the complexity of this analysis the results show that the
presence of 8-azaguanine between the second and third fission after ribonuclease treatment resulted in an inhibition of the re-appearance of metagons. Metagon synthesis would normally take place during that period.
DISCUSSION

The experiments reported in Section 1, Part 1, showed that the nu particles in mate-killer Stock 540, Variety 1 of Paramecia aurelia disappeared following the replacement of the dominant determining gene(s) N, and/or n, by the recessive allele(s) m, and/or n,2. However, it was also shown that the critical fissions as far as the disappearance of nu particles was concerned were those between the eighth and the fifteenth following the genic change. At the eighth fission stage 5% of the daughter cells had lost their nu particles and were therefore sensitive to mate-killing. At the subsequent fissions an increasing proportion of cells were found to lack the particles. It was shown in Section 2, Part 2, that the disappearance of nu particles occurred in the same manner under various environmental conditions of growth rate and temperature. One possible explanation of the results was that the nu particles ceased replication and were then diluted out following the genotypic change. At the eighth fission sensitive cells without nu particles might be expected to appear as well as cells with different numbers of nu particles. That this explanation was not valid was shown in Section 2 by the observation that a large number of nu particles were found in the few mate-killer paramecia as late as the 'fifteenth fission' stage. Another possible factor influencing the loss of nu particles was the break-up of the macronucleus which occurs during conjugation or autogamy (all our results are obtained after conjugation or autogamy—see "Methods"). However, it was shown in Section 3 that at the stage when nu particles were disappearing, in 8% of the paramecia scored, i.e. at the 'eighth fission' stage, only two out of ten animals, i.e. 20% had one macronuclear fragment.
It was also shown that the behaviour of the fragments was influenced by the environmental conditions which were studied, but the loss of mu particles was independent of such variables.

It was therefore necessary to construct a new hypothesis to explain the results. It was supposed that in addition to the visible mu particles there were other particulate factors in the cytoplasm of mate-killer animals. These factors were named 'metagons' and they were considered to function in the following manner during the process of maintenance and elimination of mu particles. Following replacement of the dominant genes $M_1$ and $M_2$ by their recessive alleles in mate-killer animals, the metagons were assumed to be distributed passively, without dividing, to the daughter paramecia. They remained active, however, and if we assume an initial number of a thousand metagons in the original conjugant paramecia, at the 'tenth fission' stage after the genetic change paramecia possessing a single metagon would occur. The crux of the hypothesis is that although these paramecia possess only one metagon they have a large number of visible mu particles. From the 'tenth fission' stage onwards animals without metagons would occur in increasing proportions, would lose their mu particles and become sensitive to mate-killing. In some clones, depending on the fluctuations in the distribution of the metagons, sensitive cells without mu particles would be expected to appear earlier or later than the 'tenth fission' stage. This explains why sensitive cells were found at the 'eighth and ninth fission' stages. The metagon hypothesis explains satisfactorily the increasing proportions of paramecia without mu particles following the genetic changes.

A further conclusion from this hypothesis was that at the 'fifteenth fission' stage, since very few mate-killers were observed (7% - Table 1)
then these few would be expected to possess one or only a few metagens as well as many mu particles. If these animals could be selected out and allowed to divide several times, then, if the 'metagon' did exist, only one of the daughter cells would contain mu particles and be classified as mate-killers. This was what was found from the results in Section 4. The existence of the metagon as a unit whose loss resulted in the change from mate-killer to sensitive following genic change was thus shown. The change of cell type could not be due to reduction in amount of some quantitative determinant below a critical threshold. If this were the explanation then groups of 0 mate-killers 8 sensitves would be expected to occur. This class was not observed.

At each fission of a 'fifteenth fission' stage animal containing one metagon, both daughter animals were initially found to contain large numbers of mu particles. During the course of the next inter-fission period all the mu particles were destroyed in the animal not containing the metagon, whilst in the sister animal with the metagon, sufficient increase in number of mu particles must have occurred to make good the loss of half at each fission (Section 4(b)). The metagenos would seem to exercise an extremely sensitive control over the mu particles. Nothing is known of the mechanism whereby this control is exerted, but it seems reasonable to suggest that the metagenos might act by inhibiting the action of some enzyme which is normally present and which is capable of destroying the mu particles.

Another property of metagenos which has been studied (Section 5(a)) was that of their distribution among daughter cells. In Section 5 it was reported that the values obtained for the various proportions of animals with and without mu particles at the 'fourteenth fission' stage deviated
from the expected values calculated on the basis random distribution of metagons from 'eleventh fission' stage mate-killers. Groups of 1 mate-killer:7 sensitives, 2 mate-killers:6 sensitives, 3 mate-killers:5 sensitives occurred in equal proportions (see Table 7). This whole phenomenon could be explained by assuming that at the earlier fission stages after the genic change, the metagons are clumped together and that the clumps begin to break-up during the later fissions. Support for this hypothesis of clumping comes from the observation of paramecia with two or more metagons at the 'fourteenth fission' stage (Section 5), and perhaps even at the 'eighteenth fission' stage (Section 4(a)). This irregular distribution might, however, be due to other causative factors — (a) a slow reproductive rate of the metagons, which would lead to an increase in number of metagons; (b) repulsion between the metagons so that they, if distributed, tend to be more evenly distributed over the cell than they would be if at random.

Further experiments are planned to discriminate between these possibilities. In view of these considerations accurate estimates of the initial numbers of metagons cannot be made at present. However, from the fact that the 50% points in the two-factor and one-factor curves (see Fig. 2) are separated by approximately one fission (page 13), it seems reasonable to conclude that the animals of genotype $M_1 m_1 M_2 m_2$ liberate twice as many metagons as animals of genotype $m_1 m_1 M_2 m_2$ or $M_1 m_1 m_2 m_2$ and further, that the metagons corresponding to the gene $M_1$ behave independently of those corresponding to $M_2$.

The increase in numbers of mate-killers following induced cytoplasmic exchange (Section 5(a)) was investigated further in Section 7. The results
are interpreted to mean that cytoplasmic exchange was responsible for counteracting the non-random segregation of the metagons which has been shown to occur in Section 5. This would explain the occurrence of a greater number of paramecia with mu particles in the experimental series than were found in the control series where non-random segregation occurs. This experiment does not tell us if cytoplasmic mixing brings about its effect by breaking up metagon clumps or by inducing metagon replication or by any other method.

Our results in Section 8 show that re-introduction of the dominant gene $N_2$ into 'fifteenth fission' stage animals with mu particles and at least one metagon, re-established the capacity to maintain mu particles in several of the daughter paramecia. There was a delay of one fission between re-introduction of the gene $N_2$ and its manifestation. It is suggested that this manifestation of the gene $N_2$ is in fact the synthesis of metagons, since when mu particles are found the metagon hypothesis demands the presence of at least one metagon. These results are not in agreement with those in Variety 4 (Sonneborn, 1943, 1945) and Chao (1953) who found a large delay for gene manifestation - eight fissions. Their experiments, however, were concerned with the doubling of numbers of kappa particles following a genotypic change from Kk to KK, not the fundamental ability to maintain kappa particles. They were unable to obtain maintenance of kappa particles if the gene K was re-introduced into kk clones which had passed the 'fifth fission' stage. It is possible, however, that maintenance of kappa particles is more readily disturbed by environmental means than maintenance of mu particles (Yeung, unpubl.).

The next part of this discussion will be concerned with the actual nature of the metagon - its relation to the determining genes and
mu particles. In the final section the chemical make-up of the metagon will be discussed.

The most likely explanation of the nature of the metagons would be to assume that they originate from the macronucleus during the break-up of this organelle following conjugation or autogamy. These fragments might contain a dominant gene ($M_1$ or $M_2$) which is still physiologically active but unable to replicate. The high degree of stability of the metagons is in conformance with this view, as is also the finding that animals containing two dominant genes ($M_1$ and $M_2$) liberate approximately twice as many metagons as animals containing only one gene. Further, it has recently been reported by Woodward, Gelber & Swift (1961) that, based on photometric measurements of DNA, the macronucleus of *P. aurelia* contains about 860 haploid sets of chromosomes. This would imply that the heterozygote $M_2M_2$ contained 430 $M_2$ genes. Such a number is roughly of the same order as the number of metagons estimated by Dr. E.C.R. Reeve to occur, but all these calculations are liable to errors.

Some support for the view that it is possible for genes to continue their physiological functioning whilst no longer able to replicate is supplied by the phenomenon of abortive transduction known in bacterial genetics. Stocker, Zinder & Lederberg (1953) found evidence in *Salmonella* that fragments of genetic material bearing a gene for motility could be introduced into a recipient bacterium lacking such a gene, but that integration of the fragment into the linkage group of the recipient did not always occur. The fragment might then be transmitted at each cell division to only one of the daughter cells, but was nevertheless able to confer motility on that cell, and this process could continue for a number of fissions. A similar 'unilinear' transmission of motility in *Salmonella*
was also found to take place with some spontaneous motile variants (Lederberg, 1956; Quadling & Stocker, 1957). Again, Ozeki (1956) obtained abortive transduction of wild-type alleles to purine-requiring mutants of *Salmonella typhimurium*. When grown on the appropriate medium, minute colonies were found containing only a single cell with the wild-type allele, which again was transmitted without replication to one daughter cell, and this process could continue for at least eighteen cell divisions.

In spite of these analogies, the evidence for the 'metagon' being a 'non-replicating' gene is not convincing, and in fact, a different explanation is now considered to be more likely. Although a single metagon is able to support as many mu particles as are ordinarily present with a full set of a thousand or so metagons, there are no indications that animals containing only a small number of metagons are under certain conditions weaker in mate-killing activity - taking a longer time to kill - than animals containing many metagons. This is the conclusion we have made from our results in Section 5(b). This would imply, if the metagons were chromosomal fragments bearing genes, that a single dose of a dominant allele (\(M_1\) or \(M_2\)) would prevail over a thousand or so recessive alleles (\(m_1\) or \(m_2\)) in the macronucleus. Such a degree of dominance would seem to be very improbable. In this connection it should be recalled that Sonneborn (1954) found that the gene \(K\), when present in the micronucleus but absent in the macronucleus (being replaced there by the recessive allele \(k\)), was unable to maintain the kappa particles. Hence, in some respects at least the metagons seem to have a greater physiological activity than genes.

It should also be remembered that there is no correlation between the
metagons and the visible macronuclear fragments which presumably contain the genes of the disrupted macronucleus after conjugation or autogamy. There are about sixty macronuclear fragments per animal at first, as compared with the estimated thousand or so metagons, and the macronuclear fragments are markedly reduced in number by starvation, whereas the number of metagons is unaffected by any environmental variations so far studied (Section 2). It is certain that when single metagons are being distributed to daughter cells at the 'fifteenth and later fissions' after loss of the genes $M_1$ and $M_2$, there are no visible macronuclear fragments present.

For these reasons and for some discussed later, it is considered that the metagon is a gene product which functions in the cytoplasm to maintain the growth of $mu$ particles. There is no unequivocal evidence for this conclusion, but we will now discuss briefly the current notions of genes and their products. After this we will describe our experiments on the chemical nature of the metagon and will show how this bears on the question of metagons as gene products.

The genes in the nucleus have been shown to be concerned with all the phenotypic characters studied so far in many different organisms. There is now on hand evidence showing that the genes work to control these characters by determining protein structure and function. It is further believed that the substance in the gene responsible for this property is deoxyribonucleic acid (D.N.A.). Substantial evidence has also been provided to show the participation of ribonucleic acid as an intermediary in this D.N.A. - PROTEIN determination. One view is that the deoxyribonucleic acid of the gene produces a 'messenger ribonucleic acid' which passes from the nucleus to the cytoplasm. This 'messenger R.N.A.' is short lived, i.e. unstable
and has a nucleotide composition resembling D.N.A. (Brenner, 1961). When this ribonucleic acid fraction arrives in the cytoplasm it transmits some particular 'information' from the gene to the particulate bodies called ribosomes. These bodies are also composed of ribonucleic acid and once the 'information' has been transferred there, the 'messenger' ribonucleic acid is destroyed. From this point on, the formation of protein is carried out in the ribosomes.

These considerations will now be shown to be relevant to questions of the nature of the metagon, since we consider the metagon to be a body intermediate between the genes (\(M_1\) and \(M_2\)) and a character (mu particle maintenance). It is however at present unknown whether a specific protein is involved in the process whereby the mu particles are maintained by metagons.

In Section 1, Part 2, we showed that the mu particles were eliminated from mate-killer cells by exposure to ribonuclease. It would seem feasible to suggest three mechanisms leading to mu particle disappearance in terms of the 'metagon hypothesis': (a) ribonuclease eliminated the mu particles without affecting the metagons; (b) ribonuclease eliminated the metagons and therefore indirectly the mu particles; (c) ribonuclease affected the elimination of both metagons and mu particles by its action on both separately.

That situation (a) is not the explanation is shown in Section 3, Part 2, where our results leave no doubt that mu particles remain viable after ribonuclease treatment. Quite clearly from Section 2, Part 2, 'seventh fission' stage paramecia were shown to possess metagons which were eliminated or inactivated by ribonuclease. In fact it is on the metagons that
ribonuclease acts to bring about the disappearance of the mu particles. Situation (b) explains the disappearance of mu particles after exposure to ribonuclease.

The failure of ribonuclease to have any effect on the mu particles by direct action is presumably connected with the fact that the mu particles appear to consist largely of D.N.A. (Beale & Jurand, 1960). What R.N.A. there is in each particle would not be an essential reproductive component and its loss would be repairable.

After elimination of the metagons by RNAase treatment the mu particles remain present for times of between 2-6 days in the cytoplasm of the treated cell. Evidently whatever the mechanism is whereby the metagon supports the growth of the mu particles it is still in operation. It is only when the cell divides that the mu particles can no longer be supported and are quickly destroyed. It appears that some particular physiological condition operates to bring about elimination of mu particles and this condition operates only during - or after - cell division. This whole phenomenon of mu particle elimination needs fuller investigation.

So far we have been considering the effect of the enzyme ribonuclease on the metagons and the resultant effect on the mu particles. However, striking changes occur in the cell if after exposure to the enzyme the gene $N_2$ is present in the nucleus. A fresh population of metagons was synthesised in $N_2$ cells following the second fission after treatment (Section 4, Part 2). The cells at this 'second fission' stage had no mu particles but had metagons. In this respect it is of interest to recall the results in Section 3, Part 2, where the proportions of
mate-killers at later fissions could be interpreted, on the metagon hypothesis, to show that the sensitive Stock 540 \((M_1, M_2)\) must have possessed metagons but not mu particles. This is thought to be so since the RNAase-treated animal had its metagons eliminated. However, the results in this Section 3, do not show directly the existence of metagons.

From the results in Section 5 it is clear that by increasing the concentrations of the enzyme and also times of exposure, the proportions of paramecia with mu particles at later fissions are affected. In terms of the metagon hypothesis this would mean that the numbers of metagons affected is also greater the longer the exposure time or greater the concentration. These results do not conflict with an interpretation involving a 'direct hit' or first order form of reaction between enzyme and metagon but this conclusion must be considered to be tentative only, in view of the small amount of data. Further experiments to clear up this problem are planned. In two systems, Bacillus megaterium and onion root cells, soluble RNA but not the other RNA fractions has been reduced quantitatively after exposure to ribonuclease (Brachet and Six, 1959; Robin, 1962). On the other hand it cannot be excluded that the ribonuclease may act indirectly, for example by releasing some other enzyme which then eliminates the metagons. It is also possibly relevant here to point out that ribonuclease not only acts on RNA substrate but has shown to form a complex with D.N.A. (Hakim, 1956). Further evidence on this issue is needed.

The work reported here has led to several interesting conclusions. By using RNAase it is possible to distinguish between the metagons and the mu particles, since the latter are not directly affected by its action. This reinforces our belief in the reality of the metagons. The enzyme also has an analytical value in that it is possible to eliminate all the metagons
and to follow the regeneration of the metagons in the presence of gene \( M_2 \).

8-azaguanine - considered to be an analogue of the nucleotide guanine which occurs in both deoxyribonucleic acid and ribonucleic acid - is interesting in that it acts in exactly the converse way to ribonuclease. It acts quickly, within six hours, to eliminate the mu particles but does not affect fully formed metagons. 8-azaguanine has been shown to be incorporated into RNA to a large extent - 40% - during synthesis of RNA (Mandel & Markham, 1958) and into DNA to a lesser extent (Smith and Matthews, 1957). Perhaps the incorporation into the DNA of the mu particle is enough to bring about its destruction. 8-azaguanine has also been shown to be incorporated into ribosomes during active bacterial cell synthesis (Otaka et al. 1962) and also into the soluble RNA of \( B. \) cereus when RNA synthesis is occurring (Chantrenne, 1958). In our experiments the analogue interferes with metagon synthesis. Since 8-azaguanine is considered to interfere with RNA synthesis (for fuller discussion see Chantrenne, 1961) our results on the inhibition of metagon synthesis support the view that RNA synthesis is necessary for metagon formation and may be taken as suggestive evidence for the presence of RNA in the chemical structure of the metagon. Further evidence for this is provided by the results with ribonuclease, particularly the suggestive 'direct hit' effect. The author feels that the evidence is fairly strong for RNA being a component of the metagon but future work must be directed towards clearing up this problem.

The relationship between genes, the metagons and mu particles has, we feel, been shown to be a case of gene determination of mu particle maintenance.
via a ribonucleic acid intermediate - the metagon. It will then be clear that our problem of gene control of mu particle growth is in fact more generally the problem of gene action. In this respect we are in agreement with the current notions of gene determination of character formation via a ribonucleic acid component as mentioned earlier (page 50). The metagon however, unlike 'messenger' ribonucleic acid is a stable entity and we plan experiments utilising this property in order to determine exactly the nature of the metagon. In respect of stability the metagon resembles the ribosomes in rabbit reticulocytes, since it has been shown that ribosomes from the rabbit continue to make haemoglobin specific to this organism after incubation with fractions from an altogether different mammal - the mouse (Bishop, Favelukes, Schweet & Russell, 1961).
After the completion of the work described above further experiments were undertaken. Attempts were made to extract intact metagons from mate-killer paramecia and to re-infect them into other mate-killer cells. The following is an account of experiments carried out towards this end. Mate-killer paramecia (genotype $M_1 M_2 M_3 M_4$) with mu particles were grown in a 2 litre flask of lettuce medium to a density of 1,000 animals/ml. This volume of animals was then concentrated to 4 ml, 1 ml. of a suspension of bentonite (1 mg./ml.) was added and then 8 ml. of a solution containing sucrose, potassium bicarbonate, magnesium chloride and potassium chloride. This mixture was homogenized by ultrasonic vibrations. The crude homogenate was centrifuged at 10,000 g. for ten minutes. The sediment was rejected and the supernatant centrifuged again at 10,000 g. for ten minutes. The supernatant was then spun down at 30,000 g. for twenty minutes and the resultant sediment rejected. A final spinning was carried out at 105,000 g. for one hour but this time the sediment and supernatant were kept. To the sediment was added 5 ml. of the solution containing sucrose etc. and 5 ml. exhausted lettuce medium. The supernatant was treated in the same way.

2 ml. portions from both sediment and supernatant mixtures were added at $18^\circ$C to drops of exhausted lettuce fluid each containing 50 paramecia at the 'eleventh fission' stage. The animals were left in these mixtures for ten hours. The survivors (90%) were isolated individually into lettuce medium and each allowed to undergo three asexual fissions. At this stage animals were scored for mu particles.
Results with sediment and supernatant fractions

In accordance with the results in Section 1, Table 1, the majority of "eleven fission" stage cells were sensitives (61%) and this is the interpretation of the thirty groups of eight paramecia found to be all sensitives (60%) in experiments with the sediment. In the other 40% of cases, however, all eight paramecia (20 groups) scored had mu particles. This is firm evidence for the infection of metagons from the sediment into "eleven fission" stage animals. The metagons must have been present in the sediment at 105,000 g. Our experiments with the supernatant however, yielded groups of eight paramecia comprising 1 mate-killer : 7 sensitives, etc. (see page 21), but none in which all eight contained mu particles. The metagons do not appear to occur in the supernatant fraction and are concentrated in the sediment.

In the future experiments will be directed to characterising the metagon further by examining this infective fraction. It would also be interesting to attempt to count the number of metagons which are re-infected.
SUMMARY

1. The work described in this thesis is concerned with the manner in which the genes $M_1$ and $M_2$ support the growth of the $\mu$ particles in the cytoplasm of mate-killer paramecia.

2. When the dominant genes $M_1$ and $M_2$ are replaced by their recessives $m_1$ and $m_2$ in mate-killer Stock 540 of Paramecium aurelia some of the daughter cells lose their $\mu$ particles and are converted from the mate-killer to the sensitive phenotype. The change occurs at some stage between the eighth and fifteenth fissions following the loss of the dominant genes. Data are presented for the proportions of mate-killers and sensitive cells at these various fission stages.

3. The appearance of cells lacking $\mu$ particles is shown to be independent of various environmental variations and also of the behaviour of the macronuclear fragments.

4. The metagon hypothesis is proposed to explain the elimination of $\mu$ particles. Metagons are considered to be gene-derivatives, non-replicating and to have the physiological activity of the $M_1$ and $M_2$ genes in that they maintain the growth of the $\mu$ particles in mate-killer cells.

5. At late fission stages it is shown that some mate-killer animals lacking the dominant genes, possess only one metagon which is sufficient to maintain the growth of many $\mu$ particles. This metagon is then transmitted in a unilinear manner at subsequent cell fissions.

6. Distribution of metagons to daughter cells (following loss of the dominant genes) is not entirely at random. It is suggested that metagons may clump together.
7. By inducing cytoplasmic exchange between mate-killer and sensitive conjugants it is found that metagons as well as mu particles can be transmitted across the cytoplasmic bridge, with the result that the sensitive mate and some of its daughter cells become mate-killers.

8. Re-introducing a dominant gene ($M_2$) into a cell with the recessive gene succeeds even after fifteen fissions in re-establishing the ability to support growth of mu particles (if any of the latter still remain). There is a lag of one fission between introducing the gene and its phenotypic manifestation.

9. The enzyme ribonuclease eliminates or inactivates the metagons but does not directly affect the mu particles. However, the latter disappear as soon as the enzyme-treated cell divides.

10. Following treatment of paramecia possessing the gene $M_2$ with ribonuclease, metagons are re-synthesised between the second and third fissions following treatment.

11. Variations in concentration and in time of exposure to the enzyme have a quantitative effect on the metagons.

12. 8-azaguanine destroys the mu particles directly but does not affect the metagons already present. However, 8-azaguanine prevents the synthesis of new metagons.

13. The relevance of the metagon to problems of gene action is discussed.
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