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The action of ribonuclease and 8-azaguanine on mate-killer paramecia

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1. INTRODUCTION

In an earlier paper we gave evidence for the existence of the cytoplasmic particles called 'metagons' in mate-killer paramecia (Gibson & Beale, 1962). In brief, the metagons were considered to be derivatives of certain genes (M_1 and M_2) whose presence was necessary for the maintenance in the cytoplasm of the symbiotic bacterium-like mu particles and therefore for the mate-killer phenotype (Gibson & Beale, 1961). Certain favourable circumstances made possible the demonstration of metagons in this material. Thus, after the dominant genes M_1 and M_2 were removed from mate-killer cells by appropriate crosses and autogamy, it was found that there was a surprisingly long period, lasting for at least eight fissions and sometimes as long as eighteen, during which cells containing large numbers of mu particles continued to be produced. Even when the dominant genes had been removed eight or more fissions previously, the visible manifestation of genic activity continued in some cells. This 'posthumous' genic effect was considered to be due to the fact that initially many (*ca.* 1000) metagons were present in each cell, that they were stable and as a rule non-replicating, and that they continued to exercise their control over the cellular characteristics long after the dominant genes had disappeared. There was a convenient period of eighteen fissions during which experiments could be carried out with the metagons.

As a result of fission of cells lacking the dominant genes, the metagons originally present were redistributed amongst the progeny until a stage was reached when some cells contained only a single metagon. The latter was however sufficiently potent to maintain some hundreds of mu particles, and even permit their multiplication, thus ensuring the continuation of the mate-killer phenotype in some, though an ever-decreasing proportion, of the cells. When a cell containing only one metagon divided and gave rise to a daughter cell lacking one, all the mu particles were rapidly destroyed in that cell, but maintained in the sister cell containing the metagon. The mu particles are therefore remarkably sensitive and reliable indicators of the presence of even a single metagon in a paramecium.

It should be added that mu particles, which are easily observed by phase-contrast microscopy in the exudate from a crushed paramedium, contain DNA, which is diffusely distributed throughout the particles (Beale & Jurand, 1960), and possibly also a certain amount of RNA. Finally, it may be mentioned that both mu particles

and metagons were successfully transferred from one paramecium to another by means of the cytoplasmic bridges which can be induced to be formed at the end of conjugation.

We now wish to study the chemical nature of metagons. As discussed previously (Gibson & Beale, 1962) these particles may be considered either as a kind of gene 'relic', liberated into the cytoplasm by disintegration of the macronucleus at conjugation or autogamy, or alternatively, as normal gene products reflecting the specificity and physiological activity of the original genes. In the latter case metagons would constitute an intermediary stage in the process whereby a gene impresses its specific properties on the phenotypic characters of the organism. Some indirect evidence favoured this second view, but the matter could not be regarded at all as settled, in view of the slight amount of factual information available.

Knowledge of the chemical nature of the metagons would be of considerable value for deciding this problem. If they should turn out to be composed of DNA, the theory of gene 'relics' would be supported; if of RNA or protein, the theory of normal intermediary products of genic activity would be favoured. In the work now to be described, mate-killer paramecia were treated with two substances which in different ways affect RNA: (1) the enzyme ribonuclease and (2) the analogue 8-azaguanine.

Cytological and electron microscopic evidence concerning the effect of ribonuclease on living paramecia has been briefly described elsewhere (Jurand, Gibson & Beale, 1962). With adequate doses of the enzyme there is a dramatic change in the cytoplasmic components: RNA, as judged by staining reactions, is removed from both cytoplasm and macronucleus, and the fine granular structures observed in the cytoplasm by electron microscopy are largely destroyed. Nevertheless a proportion of such enzyme-treated paramecia recover and in time make good the lost cytoplasmic materials. As for the effect of 8-azaguanine on living paramecia, nothing has been previously reported, but from work with other organisms (see Chantrenne, 1961), it is reasonable to believe that RNA synthesis would be inhibited in *Paramecium*. The purpose of the work to be described is to establish the effects of these two substances on the preservation and if possible the synthesis of the metagons.

2. MATERIAL AND METHODS

The following stocks of paramecia (all belonging to *P. aurelia*, syngen 1) were used:

(1) Stock 540, the original mate-killer of genotype $M_1 M_1 M_2 M_2$ and containing mu particles;
(2) Stock 540 sensitive, genotype also $M_1 M_1 M_2 M_2$ but lacking mu particles, which were eliminated from 540 mate killers as previously described (Gibson & Beale, 1962);
(3) 'Tester 7'—a mate-killer stock of genotype $m_1 m_1 M_2 M_2$ and containing mu particles (see Gibson & Beale, 1962), and
(4) Stock 513, sensitive, lacking mu particles, genotype $m_1 m_1 m_2 m_2$.

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Many of the experiments involved treatment of mate-killer paramecia of genotype $m_1m_1m_2m_2$, recently derived by autogamy from animals of genotype $m_1m_1M_2M_2$ and containing mu particles. The expressions 'first fission animals', 'seventh fission animals', etc., will be used to denote paramecia which have undergone the given number of fissions since change of genotype from $m_1m_1M_2m_2$ to $m_1m_1m_2m_2$. To determine which clones were of the desired genotype ($m_1m_1m_2m_2$) a procedure like that described previously in detail (Gibson & Beale, 1962) was used, whereby samples of animals of each ex-autogamous clone were grown rapidly through fifteen fissions whilst other samples were held back in non-nutrient medium. Those clones which lost mu particles by the fifteenth fission stage were classified as $m_1m_1m_2m_2$ and the held-back sister animals could then be used for the main experiment.

The ribonuclease and 8-azaguanine were dissolved in a non-nutrient solution of the following composition:

0.1 M sodium citrate	20 ml
0.1 M monobasic sodium phosphate	10 ml
0.1 M dibasic sodium phosphate	10 ml
0.1 M calcium chloride	15 ml
distilled water	945 ml

The final solutions were adjusted to pH 7.1 where necessary. Treatment of paramecia with these substances was carried out by adding 1 ml. of the desired concentration of ribonuclease of 8-azaguanine to 1 ml. of a paramecium culture in a depression slide containing a known number of paramecia.

The main procedure involved classification of paramecia as mate-killers or sensitives, following the various treatments. This classification was accomplished by first starving the paramecia for 24 hours in the above-described non-nutrient solution, then squashing them on a glass slide and examining the exudate by phase-contrast microscopy. If large numbers of mu particles were seen the animals were classified as mate-killers.

Methods for bringing about conjugation, cytoplasmic exchange, autogamy, etc., were as reported previously (Gibson & Beale, 1962).

3. RESULTS

(i) *Effects of ribonuclease on stock* 540 *mate-killers*

(a) *Without starvation following treatment*

Stock 540 mate-killer paramecia (genotype $M_1M_1M_2M_2$ and containing mu particles) were treated with ribonuclease (final concentration 0.5 mg./ml.) for 12 hours at 20° C. Ninety per cent of the treated organisms died. The surviving 10% were transferred to normal culture medium at 25° C., allowed to recover and scored for presence of mu particles at various times. There was a long delay, lasting about 48 hours, before the first fission, as compared with the usual 8-hour interfission period.

It was found that the mu particles were present unchanged in all paramecia examined at any time up to the first fission after treatment. In the 8-hour period between the first and second fissions following treatment, however, there was a remarkable reduction in the number of mu particles, and by the end of this period no mu particles could be seen in any paramecia. Examination at later stages, up to fifteen fissions after treatment, showed that the loss of mu particles was irreversible.

(b) *With starvation following treatment*

Stock 540 mate-killers were exposed to ribonuclease as in the previous experiment, but the surviving organisms were placed, after treatment, in non-nutrient medium for 96 hours. The paramecia were then transferred to normal lettuce medium at 25° C., where they underwent the first fission about 24 hours later. It was found that the mu particles again remained in all paramecia up to the time of the first fission after treatment, and again disappeared during the period between the first and second fissions. Thus it was clear that the destructive action of ribonuclease on the mu particles was only apparent after a period of growth sufficient to enable the organisms to undergo one fission, irrespective of the absolute time involved.

(ii) *Elimination of metagons by ribonuclease treatment of paramecia lacking the genes* M_1 *and* M_2

About sixty '7th fission' mate-killers, of genotype $m_1 m_1 m_2 m_2$, were treated with 1 ml. ribonuclease solution (conc. 0.5 mg./ml.) for 12 hours at 20° C. The survivors (again 10%) were then transferred to normal lettuce medium. After 48 hours, by which time the animals had recovered but had not yet divided, they were mated to untreated '11th fission' animals, also of genotype $m_1 m_1 m_2 m_2$. Control matings were made between untreated '7th fission' and '11th fission' animals. Cytoplasmic exchange was induced in both sets of matings. The ex-conjugants were allowed to pass through three fissions and the groups of eight animals thus obtained were examined for presence of mu particles. In order to identify the exact origin of the ex-conjugants, antigenic markers (60G and 90G) were used, as described previously (Gibson & Beale, 1962).

It will be recalled that some 39% of cells at the '11th fission' stages are mate-killers, and that nearly all of these contain one, two or three metagons. Only 4% contain more than three metagons. The remaining 61% lack metagons and are sensitive to mate killing (Gibson & Beale, 1962). All the 'seventh fission' animals are, however, mate-killers, and should contain on the average eight or nine metagons per cell (Reeve, personal communication).

The results are set out in a diagram (Fig. 1) and show that the ribonuclease treatment had eliminated the metagons from the '7th fission' animals. In the progeny of treated '7th fission' crossed with untreated '11th fission' animals, the proportion of mate-killers is consistent with the view that all the metagons present had been derived from the untreated '11th fission' animals and none from the treated '7th fission' animals. The thirteen 'all white' clones shown in Fig. 1 are considered to

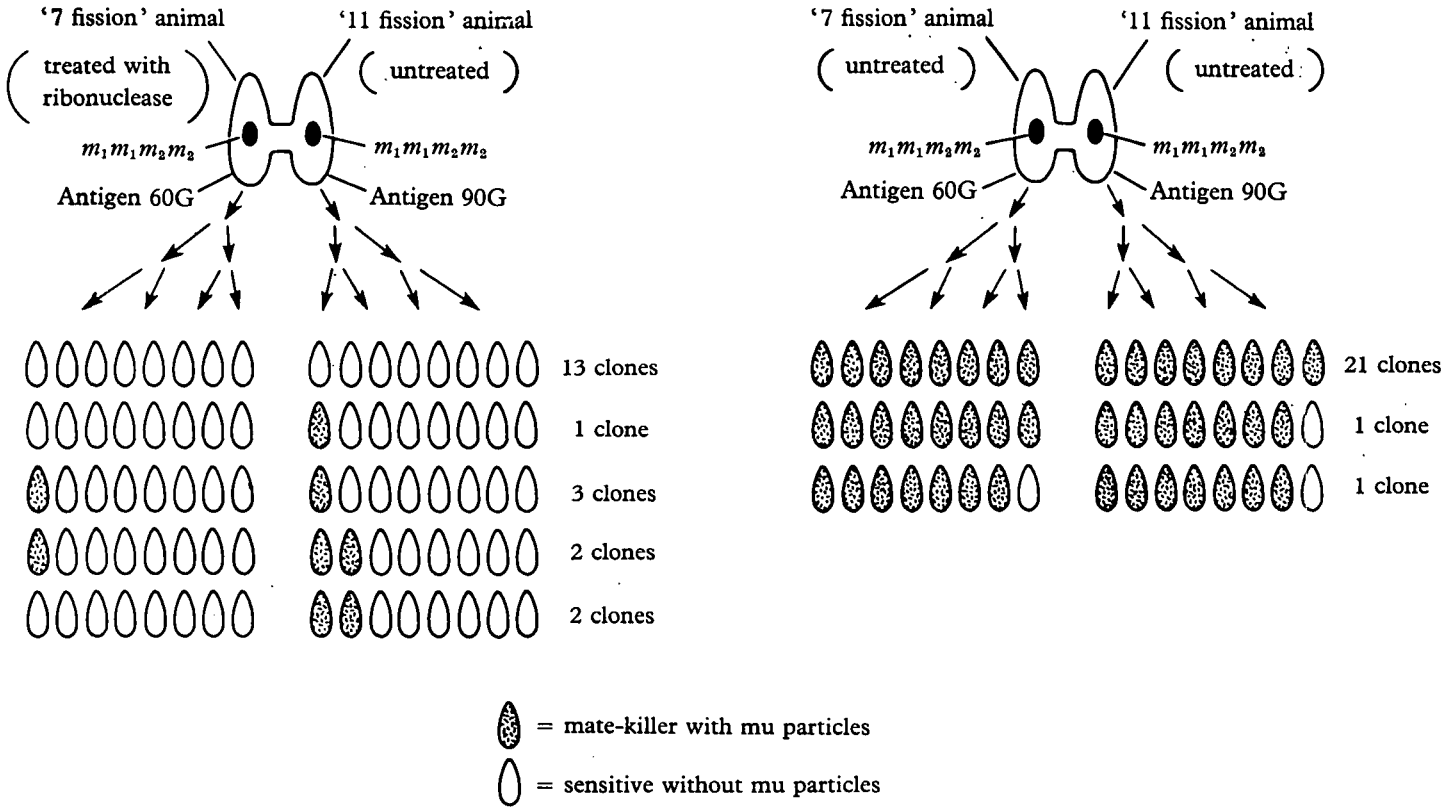

Fig. 1. Effect of ribonuclease treatment on '7th fission' animals (see text). On left, treated series; on right, untreated control series. (Antigen markers 60G and 90G were used for identification of ex-conjugants.)

be the result of matings with sensitive 11th fission animals, and as these would be devoid of mu particles their progeny would inevitably lack mu particles even if the treated '7th fission' animals retained any metagons. These thirteen clones therefore prove nothing about the effect of ribonuclease on metagons.

In the progeny of the control matings, all except three out of 184 paramecia contained mu particles, confirming that there was an adequately large supply of metagons in the '7th fission' animals, which also contributed mu particles to the matings with sensitive '11th fission' animals. It can be seen that both metagons and mu particles had passed from one conjugant to another via the cytoplasmic bridge, as described previously (Gibson & Beale, 1962).

This experiment, while clearly showing the effect of ribonuclease on metagons, did not exclude such an effect also on the mu particles directly. This matter was therefore studied by a special experiment described in the next section.

(iii) *Experiment to show that ribonuclease does not directly eliminate mu particles*

About sixty '7th fission' animals (genotype $m_1 m_1 m_2 m_2$) were treated with one ml. ribonuclease solution (0.5 mg./ml.) at 20° C. for 12 hours and the ten per cent survivors transferred to normal culture medium. Before dividing they were crossed to stock 540 sensitives, i.e. paramecia of genotype $M_1 M_1 M_2 M_2$ but lacking mu

Table 1. *Effect of conjugation, with cytoplasmic exchange, between '7th 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*

Progeny from '7th fission' mate		Progeny from stock 540 mate		No. of pairs
Mate-killers	Sensitives	Mate-killers	Sensitives	
8	0	8	0	2
8	0	7	1	1
7	1	8	0	1

particles. Cytoplasmic exchange was induced and the ex-conjugants were allowed to undergo three fissions. The resultant animals were then scored for presence of mu particles. Four pairs of ex-conjugant clones were successfully classified, and the results are shown in Table 1. It will be seen that most of the animals contained mu particles, showing that some, if not all, of the mu particles in the treated '7th fission' animals had remained viable, since the stock 540 mates did not contain any. Further, since the metagons in the treated '7th fission' animals had been destroyed by ribonuclease (as shown in the previous experiment), it is clear that the stock 540 sensitive animals had been the source of supply of metagons to the offspring. This and the previous experiment, taken together, clearly establish the separate existence of the mu particles and the metagons.

(iv) *Effect of the gene* M_2 *on the regeneration of metagons following their destruction by ribonuclease*

It was now desired to find out at what stage, if ever, regeneration of metagons caused by the activity of the gene M_2 would take place in animals from which the metagons had been removed by ribonuclease treatment. The analysis was complicated by the fact that ribonuclease caused the disappearance from the treated animals, not only of the metagons, but also, after one fission, of the mu particles. Since mu particles cannot be re-formed in a cell which has lost them, even though that cell is constitutionally able to maintain them, it was necessary to devise a procedure whereby mu particles could be re-introduced into a cell previously deprived of both metagons and mu particles, thus making it possible to determine whether or not the cell had produced a new crop of metagons. Re-introduction of mu particles was accomplished in the present experiment by inducing conjugation and cytoplasmic exchange between the animals under study and '11th fission' animals, of which, as already mentioned, 39% contain mu particles and only a small number of metagons. This procedure has the disadvantage that the re-introduction of mu particles would probably be accompanied by re-introduction of one, two or three metagons; but such small numbers of metagons would not interfere with the detection of large numbers newly produced by the dominant gene M_2, if such are in fact formed.

The scheme of this experiment involved treatment of heterozygous paramecia (genotype $m_1m_1M_2m_2$ and containing mu particles) with ribonuclease at a concentration of 0.5 mg./ml. for 12 hours, sufficient to remove all existing metagons. The survivors were then allowed to recover and pass through a known number of fissions (from one to eight), after which the descendant animals were mated to '11th fission' animals (genotype $m_1m_1m_2m_2$) with cytoplasmic exchange. Following this cross a 1:1 ratio of the genotypes $m_1m_1M_2m_2$ and $m_1m_1m_2m_2$ would be expected, but only the latter were required for the analysis, since otherwise more metagons might be formed due to the further action of the M_2 gene. Clones containing the M_2 gene (as identified by appropriate procedures) were therefore discarded.

The $m_1m_1m_2m_2$ clones obtained by crossing the progeny of the treated animals with '11th fission' animals were carried on for four post-conjugational fissions, and the proportions of cells containing mu particles then determined. The results are shown in Table 2.

Considering first the animals which were mated after only one fission following ribonuclease treatment, it will be seen that only a few of the animals finally tested contained mu particles, indicating the presence of only a few metagons, and these are assumed to be all derived from the untreated '11th fission' animal. Matings with animals which had undergone two or more fissions after ribonuclease treatment, however, yielded many mate-killer progeny in those clones which contained any at all. (The clones containing no mate-killer cells at all are assumed to have been formed from '11th fission' animals devoid of mu particles.) Thus, it is clear that new formation of metagons in $m_1m_1M_2m_2$ animals occurred between the second and third fissions following ribonuclease treatment.

Table 2. *Experiment to show time of synthesis of metagons in animals of genotype* $m_1m_1M_2m_2$, *following removal of existing metagons by treatment with ribonuclease* (*see Text*)

Stage after treatment with ribonuclease at which mating to '11th fission' animal occurs	No. of pairs showing cytoplasmic exchange	Bilateral* death	Unilateral* death	Analysis of groups of eight‡ animals obtained by four fissions of ex-conjugants				No. of clones with the indicated proportions of animals containing mu particles
				(*a*) from the 'treated' conjugant		(*b*) from the '11th fission' conjugant		
				Mu particles present	Mu particles absent	Mu particles present	Mu particles absent	
'1 fission'	12	1	2	0	8	0	8	3
				1	7	1	7	1
				0	8	1	7	1
				0	8	2	6	2
				0	8	4	4	2
'2 fission'	12	1	3	0	8	0	8	5
				7†	0	7†	0	3
'4 fission'	10	0	0	0	8	0	8	6
				8	0	8	0	4
'8 fission'	56	3	3	0	8	0	8	30
				8	0	8	0	20

* These were not further analysed.

† In these cases the 8th animal was allowed to pass through a further seven fissions (thus arriving at the '11th fission' stage). The percentage of animals with mu particles was then found to be 37%.

‡ Although the ex-conjugants from these crosses were carried on for four fissions before examination for mu particles, only groups of eight cells were available for analysis. This was because after the first post-conjugational fission, one animal from each cross was rapidly passed through about eight fissions to enable the genotype of the clone to be determined.

It would be interesting to find out whether a full set of a thousand or so metagons was produced immediately. The present experiment cannot be considered to give a definite answer to this question but, as shown in Table 2, some of the animals obtained by mating treated paramecia after two fissions were carried on for as many as 11 fissions after introduction of mu particles, by which time 37% of the progeny still contained mu particles. This is very close to the number obtained in previous work (Gibson & Beale, 1962) using as starting material animals containing a full set of metagons. Thus it seems likely that a large number, if not a complete set, of metagons is formed between the second and third fissions following ribonuclease treatment, if the gene M_2 is present.

(v) *Effects of varied concentrations and durations of exposure to ribonuclease on destruction of metagons*

Samples of 'seventh fission' paramecia were exposed to the action of ribonuclease at concentrations between 0.125 mg./ml. and 0.50 mg./ml. and for times between 1 and 12 hours. After each treatment four of the survivors were removed from the

Table 3. *Effect of ribonuclease treatment of '7th fission' paramecia on proportions of mate-killers and sensitives occurring after four further fissions. Varying concentrations of ribonuclease and varying durations of treatment were applied*

Concentration of RN-ase (mg./ml.)	Duration treatment (hrs.)	No. of animals treated	% survival	% mate-killers after four further fissions (= at '11th fission' stage)
0	—	—	—	39*
0·125	1	60	95	35
	2	50	95	34
	4	60	85	31
	6	50	75	27
0·25	1	50	98	37
	2	60	95	37
	4	50	74	28
	6	60	94	18
	8	70	90	7
	10	65	83	2
	12	60	70	1
0·375	6	50	44	11
0·50	1	60	80	35
	2	50	25	32
	4	60	18	26
	6	55	10	2
	12	2000	5	0

* Data from Gibson & Beale, 1962.

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ribonuclease solution, placed in bacterized medium and allowed to pass through four more fissions. The groups of sixteen animals which would then be at the '11th fission' stage were examined for presence of mu particles. The results are given in Table 3, and show that both increasing concentration of ribonuclease and increasing time of exposure result in an increase in the number of metagons destroyed.

(vi) *Effect of 8-azaguanine on stock* 540 *mate-killers*

Stock 540 mate-killers ($M_1M_1M_2M_2$ and containing mu particles) were placed for six hours in 8-azaguanine solution at concentrations 0.0125, 0.025 and 0.050 mg./ml. respectively. Immediately after treatment the paramecia were examined for presence of mu particles. The results are given in Table 4, and show that there is a marked destructive effect of the substance on the mu particles even without any further growth of the paramecia. The action of 8-azaguanine on the mu particles is therefore more immediate than that of ribonuclease.

Table 4. *Effect of treatment of stock* 540 *mate-killer paramecia with* 8-*azaguanine*

Conc. of 8-azaguanine solution (mg./ml.)	No. of cells examined	% amimals containing mu particles after 6 hours' treatment
0·0125	25	60%
0·025	20	0%
0·05	25	0%

(vii) *Experiment to show that the effect of 8-azaguanine is not on the metagons*

About sixty '7th fission' mate-killer animals (genotype $m_1m_1m_2m_2$ and containing mu particles) were treated with 1 ml. 8-azaguanine solution (0.05 mg./ml.) at 20° C. for 6 hours. The surviving animals (25%) were transferred to lettuce medium, were found to have recovered after 48 hours and before dividing were crossed to '11th fission' animals (genotype $m_1m_1m_2m_2$). Cytoplasm was exchanged between the mates in three pairs and the ex-conjugants were then allowed to undergo three fissions. The eight daughter cells derived from each ex-conjugant were examined for presence of mu particles. The results are shown in Fig. 2.

Since the mu particles have been shown in the previous experiment to be removed by the concentration of 8-azaguanine used here, the two groups of sixteen cells all containing mu particles must have received mu particles from amongst those originally present in the untreated '11th fission' conjugant. It has already been pointed out that the overwhelming majority of '11th fission' mate-killers possess only one, two or three metagons. The number of mate-killers found in the progeny of the mated paramecia previously treated with 8-azaguanine is therefore too large to be explicable by derivation of metagons only from the '11th fission' conjugant. It is concluded that the '7th fission' cell retained some or all of its metagons after treatment with 8-azaguanine, even though all the mu particles were destroyed. It

is assumed that the one group of sixteen 'all sensitive' cells shown in Fig. 2 was the result of a cross between a treated '7th fission' cell and an untreated '11th fission' cell lacking mu particles.

Hence, treatment of mate-killer cells with 8-azaguanine resulted in direct elimination of mu particles but had no apparent effect on the metagons present. The mechanism of 8-azaguanine action thus differed strikingly from that of ribonuclease though the end result—loss of mu particles—was the same.

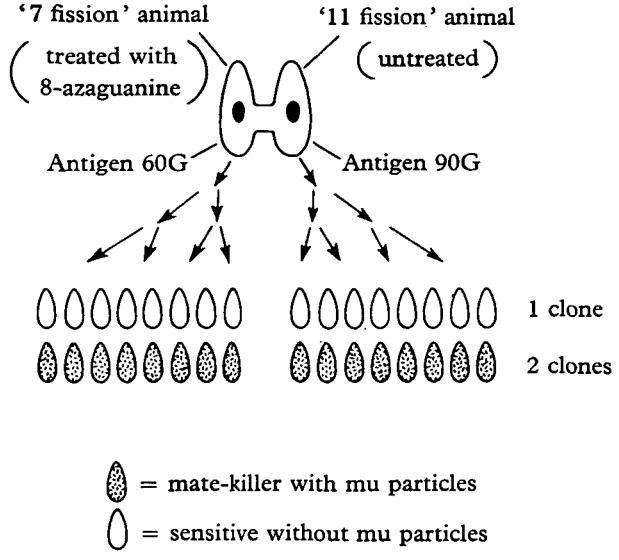

Fig. 2. Analysis of groups of 8 animals obtained following conjugation of '7th fission' animals (previously treated with 8-azaguanine) and '11th fission' animals, with induced cytoplasmic exchange.

4. DISCUSSION

Both ribonuclease and 8-azaguanine have been shown to affect mu particles: ribonuclease destroys or irreversibly inactivates the metagons; 8-azaguanine does not affect 'mature' metagons (though it is possible that it inhibits synthesis of new ones.) In addition, ribonuclease has no immediate destructive effect on the mu particles, while 8-azaguanine does. The experiments on the action of these two substances on living mate-killer paramecia have yielded results strengthening the metagon hypothesis. In particular they have clearly confirmed the separate existence of metagons and mu particles.

The effect of ribonuclease on the metagons, as revealed by the experiments described in this paper, should be considered in conjunction with the cytological observations described elsewhere (Jurand, Gibson & Beale, 1962). There it was shown that ribonuclease removed RNA from the cytoplasm and macronucleus, and by electron microscopy a destruction of fine granular material in the cytoplasm was seen. Moreover, these components were regained at a time coinciding remarkably

well with that at which the formation of a new set of metagons takes place, namely between the second and third fissions after ribonuclease treatment. Thus RNA, fine granules and metagons disappear and re-appear at the same time.

It is interesting to consider the exact sequence of events following ribonuclease treatment. Immediately after treatment the metagons have disappeared but the mu particles remain unaffected, and this state of affairs continues until the next cell division, no matter how long this may be delayed. Once cell division has taken place, however, conditions in the cell change in such a way as to destroy all the mu particles present. No satisfactory explanation can be offered at present to explain the dramatic change which occurs at, or shortly after, the time of fission of a cell from which metagons have been removed. As described previously (Gibson & Beale, 1962) a similar abrupt change occurs when cells containing only one metagon divide, yielding one daughter cell with and one without a metagon. The latter cell becomes suddenly unable to tolerate mu particles any longer.

Between the first and second fissions following ribonuclease treatment the paramecia contain neither metagons nor mu particles, and are deficient also in pyronin-staining material and fine granules in the cytoplasm. Between the second and third fissions after treatment, however, the cells return to normal in regard to cytological composition, and also—assuming a dominant *M* gene is present—form a new set of metagons capable of maintaining mu particles. However, the mu particles do not reappear, since they were all destroyed before the second fission and cannot be formed *de novo*. This very precise timing (in terms of cell division) of disappearance of metagons and mu particles and re-appearance of metagons explains the fact that treatment of stock 540 mate-killers (genotype $M_1M_1M_2M_2$) with ribonuclease results in an irreversible loss of mu particles.

The relations between concentration of ribonuclease and proportion of metagons eliminated, and between duration of exposure to ribonuclease and proportion of metagons eliminated, cannot be interpreted with certainty at present. From the increased inactivation of metagons with higher doses of ribonuclease, it would appear that the amount of enzyme acting on the metagons is limiting, and in view of the high external concentration of enzyme used, this seems surprising. However we have no information about the amount of enzyme which is available inside the cell for action on the metagons. Furthermore, it should be remembered that the metagons here considered, assuming they consist of RNA, would amount to a very small fraction of the total RNA in a paramecium, for there would of course be other kinds of RNA present in the cell as substrate for ribonuclease.

Turning now to 8-azaguanine, the action of this analogue on paramecia is much less clear than that of ribonuclease. In particular it is not understood why the mu particles, which so far as is known, are mainly DNA-containing structures, should be directly destroyed by treatment with 8-azaguanine, which is generally considered to be incorporated into RNA but only slightly into DNA (see Chantrenne, 1961). Taking all the results together, however, the present indications are consistent with the view that RNA is an essential ingredient of metagons, though in view of the complexity of the conditions within a living paramecium it would be rash to accept

this view as final. The presence of DNA in metagons cannot at present be considered to have been excluded. In future work, purified preparations of metagons derived from mate-killer paramecia will be studied outside the organisms and used to re-infect new paramecia. Under these conditions a much greater degree of precision should be attainable and make possible an exact chemical characterization of the metagons.

5. SUMMARY

1. Mate-killer paramecia (derived from stock 540, *P. aurelia*) were treated with ribonuclease and 8-azaguanine to determine the effect of these two substances on the mu particles and metagons.

2. Ribonuclease destroyed the metagons in living paramecia but had no direct effect on the viability of the mu particles. The latter were however destroyed precisely after one fission of cells whose metagons had been eliminated by ribonuclease treatment.

3. Re-synthesis of metagons was found to take place between the second and third fissions after treatment with ribonuclease, if the dominant gene M_2 was present.

4. Increasing the concentration of ribonuclease in the external medium, or in the duration of exposure of paramecia to ribonuclease, resulted in destruction of increasing proportions of metagons.

5. 8-azaguanine did not destroy metagons already present, but destroyed the mu particles immediately.

6. It is concluded that the results obtained with these two substances are consistent with the view that RNA is an essential constituent of metagons.

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