TECHNICAL PAPERS

Nitrogen Mustard Inactivation of the Cytoplasmic Factor Kappa, in *Paramecium aurelia*, Variety 4[1]

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The growing importance of chemical mutagens and their use in experimental genetics make a study of their effects on cytoplasmic factors desirable. The present work is concerned with the effects of the nitrogen mustard, methyl bis(ββ′-dichloroethyl)amine, on the cytoplasmic factor kappa, in *Paramecium aurelia*, variety 4, stock 51.

The first experiments were designed to test the effect of nitrogen mustard on killer animals growing normally at 27° C. In experiment 1, 2 ml of a freshly prepared buffered solution (phosphate, pH 6) of the hydrochloride salt of nitrogen mustard in a concentration of 0.19 mg/ml was added to a Columbia dish containing approximately 1000 killer animals in a small drop of culture fluid. Samples of up to 50 animals were washed and the animals were isolated individually and allowed to multiply for several days at maximum fission rate. At the end of this growth period, the cultures were tested for the presence of animals sensitive to the action of paramecin, the antibiotic which is produced by killer animals and which is responsible for their killing action. The method for the determination of the character of a culture is as follows. Samples of the culture in question are mixed separately with known killer animals and with known sensitive animals. If killing occurs in the mixture with killer animals, the culture contains sensitive animals. If killing occurs in the mixture with sensitive animals, the culture contains killer animals. If killing occurs in both mixtures, both sensitive and killer animals are present in the culture. In this case, the culture in question would show autolethality, i.e., the sensitive animals in the clone would be killed by the killer animals present. This method for testing the character of a culture was used throughout the study.

Experiment 2 was performed in the same manner as experiment 1. The exposing concentration was 0.434 mg/ml and different time intervals for exposure were used. Both experiments are summarized in Table 1.

It may be seen from this table that some of the killer animals were changed so that they gave rise to sensitive progeny. It will also be noted that, in general, the heavier the exposure to nitrogen mustard, the greater the proportion of clones containing sensitive animals and the greater the proportion of completely sensitive clones produced. Mortality also parallels the length of exposure. Killer animals not exposed to nitrogen mustard fail to produce sensitive progeny under similar conditions.

One interpretation of these data is that kappa has been inactivated in varying amounts with the different exposures. The production of sensitive animals, therefore, means that the number of kappa particles remaining in an animal is small enough to allow the animals to become sensitive to paramecin action (*2*). Animals which, at fission, receive at least one particle of kappa are able to give rise to clones which contain killer animals because kappa, when not affected by nitrogen mustard, can multiply more rapidly than the animals under the conditions of the experiments. Animals receiving no kappa are unable to initiate kappa formation and so remain sensitive (*2, 5, 6*). That this is the correct interpretation is shown by the experiments described here.

TABLE 1

EFFECT OF NITROGEN MUSTARD ON ANIMALS GROWN AT 27° C

Expt.	Length of exposure (hr)	No. isolated	Total No. tested*	Fraction of total tested containing any sensitive animals	Fraction of total tested containing only sensitive animals†
1	0.167	40	40	0.05	0.00
1	0.250	46	46	0.02	0.00
1	0.500	49	49	0.43	0.00
1	1.000	42	42	0.43	0.00
1	2.000	50	50	0.56	0.00
1	4.000	49	49	0.12	0.00
1	8.000	49	19	0.68	0.32
2	1.000	39	38	0.95	0.03
2	2.250	38	38	0.97	0.34
2	4.000	39	31	1.00	0.32
2	6.250	38	16	1.00	0.75
2	24.000	40	1	1.00	0.00

* Differences in the number isolated and the number tested are due to mortality.

† Clones containing only sensitive animals do not regain killing ability even when grown at one fission per day for three weeks. These clones, then, may be considered as having been derived from animals in which kappa was completely destroyed.

The expansion technique (Sonneborn, unpublished) allows the mean kappa particle number of the progeny of a killer animal to be reduced to any desired level. This technique involves growing the animals at 33.8° C, at which temperature the number of kappa particles fails to increase, and the animals undergo rapid fission, thus decreasing the number of kappa particles within the animals at each fission. The period of rapid fission at 33.8° C is followed by a period of slow fission rate at 27° C,

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at which temperature kappa can multiply faster than the animals. Those which contain at least one particle of kappa produce progeny with the full number and so yield killer clones. Those containing no particles remain sensitive and yield sensitive clones. This provides a means for calculating the number of kappa particles present in the original killer animal. The number of kappa particles present, on the average, in the progeny of a killer animal subjected to the expansion technique may be calculated by the approximate relation $P_0 = e^{-m}$, where P_0 is the proportion of individuals with no particles, m is the mean number of kappa particles per animal, and e is the base of natural logarithms (*1*).

If inactivation of kappa particles has occurred in animals exposed to nitrogen mustard, one can measure the extent to which this has occurred by comparing the mean number of particles in the progeny of exposed animals to that in the progeny of normal killer animals subjected to the expansion technique.

This comparison between animals exposed to nitrogen mustard (0.20 mg/ml) and animals exposed to buffer alone (controls) for a period of 7 min was made in experiment 3. A fresh solution of nitrogen mustard hydrochloride in phosphate buffer (pH 6) was made up twice as concentrated as was desired for the exposure. One drop of this solution was used immediately by adding it to an equal-sized drop of buffer containing the animals in a depression slide. The two drops were mixed quickly by agitating the slide, and after 7 min the animals were removed, washed three times in fresh culture fluid, isolated, and placed at 33.8° C for rapid fission. The exposed animals underwent seven fissions, and the controls underwent eight fissions. The results of carrying out these procedures on seven exposed and nine control animals from the same clone are given in Table 2.

TABLE 2

EFFECT OF NITROGEN MUSTARD ON THE NUMBER OF KAPPA PARTICLES IN KILLER ANIMALS

Animal No.	No. isolated and tested	Fraction sensitive	Mean No. of kappa particles
Exposed			
1	127	0.724	0.323
2	118	0.797	0.227
3	115	0.765	0.267
4	127	0.874	0.135
5	118	0.780	0.248
6	118	0.797	0.227
7	117	0.667	0.405
			Avg 0.261
Control			
1	233	0.236	1.44
2	99	0.222	1.42
3	98	0.092	2.39
4	98	0.143	1.95
5	98	0.122	2.10
6	94	0.106	2.24
7	27	0.148	1.91
8	29	0.138	1.98
9	24	0.167	1.79
			Avg 1.91

From the proportions of sensitive animals produced in the two series shown in the table, one may calculate that the average mean particle number for the progeny of the control animals was 1.91, whereas that for the exposed animals was 0.261. Since the controls went through one more fission than the exposed animals, the control value of 1.91 particles per animal must be doubled to make the value comparable to that found in the animals exposed to the action of nitrogen mustard. The percentage of kappa remaining after exposure is then $\frac{.261 \times 100}{2 \times 1.91} = 6.8\%$.

Experiments carried out using different lengths of exposure to nitrogen mustard indicate that the longer the exposure, the greater the percentage of kappa particles inactivated.

This procedure cannot be placed on an accurate quantitative basis at the present time because the effects of temperature alone on kappa have not been thoroughly investigated. There should be a way to correct for the effects of temperature after these have been studied in detail.

The results reported here were confirmed with the aid of the light microscope, using the methods of Preer (*3*, *4*). The cytoplasm of killer animals contains Feulgen-positive particles which are not found in sensitive animals, and which parallel the behavior of kappa. The numbers of these particles exhibited by animals exposed to nitrogen mustard were much smaller than the numbers found in untreated animals. There then seems little doubt but that the staining reaction associated with kappa and its activity as regards the killer character are destroyed by the action of nitrogen mustard.

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A New Method for the Study of Submicroscopic Spaces

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Proceeding with their research on the optical properties of osseous substance, Dallemagne and Melon (*2*) attempted to obtain diagram curves of structural double refraction of the organic constituent of bone. Since the results, which will be published, have shown anomalies in the general aspect of the diagrams, we have been looking for a different technique for scanning the submicroscopic