RESPIRATORY STUDIES ON *PARAMECIUM AURELIA*, VARIETY 4, KILLERS AND SENSITIVES*§

by

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INTRODUCTION

Several systems of inheritance within the species, *Paramecium aurelia*, have been studied by SONNEBORN and his associates[3,4,5,6]. The genetic control of one of these, the killer trait, has been worked out in detail. It has been established that some stocks are phenotypically "killers" and can kill animals of the "sensitive" phenotype by releasing a poisonous substance, "paramecin", into the culture medium. This substance affects sensitive animals in a typical manner[7]. Paramecin is found in the medium from which killer animals have been removed as well as in breis of killer animals[8].

A cytoplasmic component, "kappa", controls the production of paramecin. Kappa is maintained from generation to generation by the killer gene, designated "K". Animals possessing the killer gene (either in the homozygous or heterozygous condition) and a normal complement of kappa show the killer trait. The genotype of the sensitives may be either KK, Kk, or kk. Those which have the KK or Kk genotype and lack kappa are sensitives but can be transformed into killers upon the introduction of kappa into their cytoplasm. This can be accomplished by allowing exchange of cytoplasm between sensitive and killer animals at the time of mating[9], or in other cases by exposing the sensitive animals to concentrated breis of killer animals[10,11]. Once a small amount of kappa is introduced into the cell, it will multiply under the influence of the K gene, provided that optimum conditions of feeding and temperature are maintained. The other sensitive types are those which lack both the killer gene and kappa. The genotype of these sensitives is designated kk. By breeding analysis, it has been shown that the K and k genes are alleles.

Kappa has been termed a "self-duplicating" cytoplasmic particle. The principal support for self-duplication has been obtained by DIPPELL in her studies on the muta-

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§ Preliminary reports of this work have been presented[1,2].

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bility of the killer character[12,13]. She analyzed several mutations which appeared in stocks of variety 4 killers and demonstrated that no mutation had occurred in the killer gene. The changes in killer character were strictly correlated with the presence of different types of kappa. Thus it is apparent that the specificity for the particular killer type does not reside in the nuclear genes but is determined by kappa.

The cytochemical similarity between kappa and nuclear genic material has been demonstrated by PREER, who established the presence of Feulgen positive particles in the cytoplasm of killer animals and the absence of these particles in the sensitives[14,15]. The stained particles are either identical or are closely associated with kappa. Further, the stainable particles in the killers can be removed by incubating the animal preparation with desoxyribonuclease prior to staining. The approximate size of the stained bodies is between 0.2 and 0.8 micron.

It could be expected that the maintenance of a cytoplasmic particle of the size of kappa would impose an additional metabolic requirement upon the killer cell, resulting in different metabolic patterns in killer and sensitive animals.

MATERIAL AND METHODS

I. *Stocks*

The four stocks of *P. aurelia*, variety 4, mating type 7 obtained from the laboratory stocks of Dr T. M. SONNEBORN were 51.7 killers and 51.7, 29.7 and d 186.7 sensitives. The 51.7 sensitive stock has the KK genotype and differs from the 51.7 killer only in the absence of kappa from the cytoplasm, while the 29.7 stock has the kk genotype. The d 186.7 stock is "highly" isogenic with the stock 51 and differs from it in the presence of the k gene instead of the K gene[13]. This stock was derived from a cross between a stock 29 sensitive animal and a stock 51 killer. The progeny derived from the original cross segregated into killers and sensitives following autogamy in the F-1 generation. The sensitive segregants were then back-crossed to the stock 51 killer, and the procedure was repeated. Stock d 186.7 was obtained from one of the sensitives which segregated after the seventh backcross.

II. *Culture technique*

Animals used for the respiratory studies were grown in test tubes. The culture medium consisted of a lettuce infusion prepared from dried baked lettuce[11], inoculated with a pure strain of *Aerobacter aerogenes* twenty-four hours prior to the time of addition of the paramecia. One volume of paramecia culture was added to one volume of bacterial suspension. The animals were grown for forty-eight hours during which time the maximum population density in the particular culture medium was attained. The animals usually cleared the tubes within the first twenty-four hours. Fresh culture medium was added every other day. The effective average growth rate was one-half fission per day. Larger mass cultures were grown in a similar manner. Animals used for the particular experiments were always taken from forty-eight hour cultures. Under these conditions the bacterial concentration was at a low level.

III. *Respiratory measurements*

The Cartesian diver technique as described by HOLTER[16] was used for these respiratory studies. The diver was selected in preference to the Warburg-Barcroft type respirometer for several reasons. An adequate response with the usual Warburg apparatus can only be obtained when high concentrations of paramecia are used. In order to satisfy this condition, large mass cultures would have to be concentrated with the risk of damaging the animals. In addition, the conditions in the Warburg flasks would be unphysiological with respect to population densities, and the respiratory rates might be affected by the accumulation of toxic end-products. Determinations made in the diver avoid this complication. An adequate response may be obtained with divers having a gas volume of ten microliters when ten to twenty animals in 3.2 microliters of respiring medium are used. This is a concentration of animals only slightly higher than that at which they are normally grown (*i.e.*, 3,000 to 6,000 per ml).

The arrangement of drops in the divers is shown in Fig. 1. Drop A consists of 3.0 microliters of sodium hydroxide (0.1 *N*). Drop B contains the paramecia in 3.2 microliters of respiring medium. Above this, a 3.0 microliter drop of paraffin oil, C, serves to prevent diffusion of oxygen out of the

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diver; and finally, at the top of the diver, a 2.0 mm length of head seal, D, consisting of half-strength flotation medium, seals the diver. In all experiments in which substrates or inhibitors were used the substance was added to the respiring medium by means of the side-drop technique[17]. A side drop (E, Fig. 1) was placed on the side of the diver immediately below drop B which contained the paramecia. The placing of the side drops was facilitated by coating the inside of the diver with a hydrophobic film. Organo-silicone compounds provide a satisfactory coating[18]. One of these, Dri-film 9987, was obtained from the General Electric Company. It was used as a solution of 5% Dri-film solution in chloroform. The diver was filled with this solution, immediately sucked dry, and dried in an oven at 110° for 10 hours. After drying, the coating gave a slight acid reaction. The diver was then washed with glass-redistilled water and dried.

For each determination, a control diver was filled, containing the same arrangement of drops, but no paramecia were added. After the divers were placed in the flotation vessels, a period of one hour was allowed for attaining thermal equilibrium. At this time, a high overpressure was placed on the flotation system by means of a syringe, and the liquid seals in the diver were displaced downward until the respiring medium made contact with the side drop. The time of addition of the side drops was taken as the start of the experimental period.

All of the respiratory measurements were made at 27 ± 0.02°. There was a constant rate of diffusion of gas out of the control diver which was subtracted from the rates determined in the experimental divers. All rates reported are corrected values, expressed as millimicroliters of oxygen consumed per animal-hour.

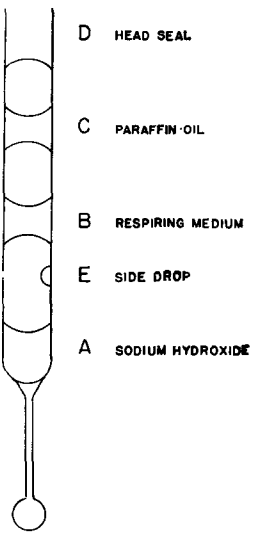

Fig. 1. Arrangement of seals for the Cartesian Diver determinations.

IV. *Respiring media*

Three different respiring media were used during the course of these investigations. Each of these preparations was adjusted to a pH of 7.0, placed in test tubes, autoclaved, and stored for future use. The pH was rechecked immediately before each experiment.

Respiring medium I consisted of autoclaved "exhausted" culture fluid. A single batch of fluid was used for all of the determinations. It was obtained from a large culture of stock 31 sensitive animals. The animals and any contaminating bacteria were removed by filtration through a Berkefeld filter.

Respiring medium II consisted of a lettuce infusion in which *Aerobacter aerogenes* had grown for twenty-four hours. The whole suspension was then autoclaved.

Respiring medium III was an inorganic salt medium in glass-distilled water, of the following composition: NaCl, 0.04%; KCl, 0.01%; $CaCl_2.H_2O$, 0.02%; $MgSO_4.7H_2O$, 0.01%; K_2HPO_4, 0.005%; and KH_2PO_4, 0.005%.

V. *Cytochrome oxidase determination*

The cytochrome oxidase activities were determined according to the method of SMITH AND STOTZ[19], using the dye, 2,6-dichloro-benzenoneindo-3′-chlorophenol as an indicator. The cytochrome C was obtained from the Sigma Chemical Company and used without further purification. The paramecia used in these experiments were grown in large mass cultures (three to six liters). The cultures were filtered through cotton, and concentrated to a volume of twenty-five ml by filtration through a Berkefeld filter. The animals were then electromigrated[20]. Respiring medium III proved to be a satisfactory migrating medium. The migrations were run for thirty minutes, and the paramecia were withdrawn from the apparatus. The population densities were adjusted to between 25,000 and 30,000 paramecia per ml by the addition of fresh migrating medium. Following this, a five ml sample of the concentrated suspension was placed in an ice bath and chilled for fifteen minutes. The suspension was then homogenized, and the cytochrome oxidase activity was determined immediately.

RESULTS

Respiratory rate. The rates of oxygen consumption of killer and sensitive paramecia were compared using Medium I as the respiring medium. Determinations were made simultaneously using killers and sensitives which had been grown under identical conditions of feeding. Thus for each value of a respiratory rate of sensitive animals reported

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in Table I, there is a corresponding value for the killer animals. In every case, the rate of respiration of the killers was much higher than that of the sensitives. The mean Q_{O_2} for the 51.7 killer stock is about 180% that of the sensitive stock used for comparison. There is no significant difference in respiratory rate among the three sensitive stocks.

TABLE I

RATES OF OXYGEN CONSUMPTION OF KILLER AND SENSITIVE PARAMECIA

Determinations were made on ten to twenty paramecia in each diver, using respiring medium I. Readings were taken at hourly intervals for a period of ten hours. The Q_{O_2} values are expressed as millimicroliters of oxygen consumed per animal-hour.

Sensitive Stocks (kappa absent)				*Killer Stock (kappa present)*			
Stock No.	*Genotype*	Q_{O_2}		*Stock No.*	*Genotype*	Q_{O_2}	
		No. of Expts.	*Mean ± S.E.*			*No. of Expts.*	*Mean ± S.A.*
29.7	kk	11	0.45 ± 0.04	51.7	KK	12	0.87 ± 0.09
51.7	KK	10	0.51 ± 0.03	51.7	KK	10	0.90 ± 0.09
d1 86.7	kk	11	0.55 ± 0.04	51.7	KK	10	0.96 ± 0.05

The possibility that the higher respiratory rate of the killer animals might have been induced by the particular respiring medium was checked in a series of experiments in which Media II and III were used. The data for the respiratory rates in all three media are found in Table II. The type of medium does not appreciably alter the ratios of the Q_{O_2} values of killer and sensitive animals. In each medium the killers respire at a rate 150 to 180% of that of the sensitive animals. The differences between the rates for the two sensitive types are not significant. It is evident, from a comparison of the rates of oxygen consumption of any particular type in the three media, that Medium I supports the highest level of respiratory activity, and that the animals respire at the lowest rate in Medium III.

The higher respiration of the killers must be caused by the presence of kappa in the cytoplasm since sensitive animals of either genotype, KK or kk show no differences in their rates of oxygen consumption. This suggests that kappa is in some manner changing the respiratory metabolism of the cell, either by alteration of the whole cellular respiratory pattern, or by virtue of its own independent capacity to carry out respiratory processes.

Carbon substrates. The effect of the addition of the carbon substrates, glucose and acetate, to the salt solution, Medium III, was tested. Glucose in a concentration of 10^{-2} *M* stimulated the respiration of the sensitive animals, but had no effect upon the respiratory rate of the killers (Table III). Acetate (Table IV) in a final concentration of either 10^{-2} or 10^{-3} *M* resulted in a slight increase of the Q_{O_2} of the killer animals, while the respiration of the sensitives was markedly stimulated.

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TABLE II

RATES OF OXYGEN CONSUMPTION OF KILLER AND SENSITIVE PARAMECIA IN DIFFERENT MEDIA

Determinations were made on ten to twenty paramecia in each diver. Readings were made at hourly intervals for a period of ten hours for experiments carried out in medium I. In the experiments in which media II and III were used, determinations were made on twenty animals. The duration of the experiments was six hours, and hourly readings were taken. The Q_{O_2} is expressed in millimicroliters per animal-hour. "Kill./Sens." represents the ratio of the mean Q_{O_2} value of killers to that of the sensitives.

Stock	51.7 *KK* (*x*) *Killer*		51.7 *KK* *Sensitive*			d 186.7 *kk* *Sensitive*		
Genes								
Type	Q_{O_2}		Q_{O_2}			Q_{O_2}		
	No. of Expts.	*Mean* $\pm$ *S.E.*	*No. of Expts.*	*Mean* $\pm$ *S.E.*	*Kill./Sens.*	*No. of Expts.*	*Mean* $\pm$ *S.E.*	*Kill./Sens.*
Medium I	35	0.90 ± 0.04	10	0.51 ± 0.03	176%	13	0.52 ± 0.04	177%
Medium II	16	0.36 ± 0.03	13	0.23 ± 0.02	157%	13	0.20 ± 0.02	180%
Medium III	8	0.24 ± 0.01	6	0.16 ± 0.02	150%	8	0.13 ± 0.01	185%

TABLE III

THE EFFECT OF ADDED GLUCOSE UPON THE RATE OF OXYGEN CONSUMPTION OF KILLER AND SENSITIVE PARAMECIA

Determinations were made using twenty paramecia per diver in respiring medium III. Glucose was added in a concentration of 10^{-2} *M* at the start of the experimental period. Readings were made immediately before and after the addition of glucose and at hourly intervals for five hours thereafter. The Q_{O_2} values are expressed as millimicroliters of oxygen consumed per animal-hour. For each rate determined in the presence of glucose a corresponding control rate was obtained at the same time.

Stock	Q_{O_2}	
	Control	*Added glucose*
51.7 killer	0.29	0.25
	0.25	0.24
	0.22	0.22
	Mean 0.26	0.24
51.7 sensitive	0.19	0.22
	0.24	0.33
	0.13	0.17
	0.12	0.16
	Mean 0.17	0.22
d186.7 sensitive	0.09	0.10
	0.16	0.26
	0.14	0.24
	Mean 0.13	0.20

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TABLE IV

THE EFFECT OF ADDED ACETATE UPON THE RATE OF OXYGEN CONSUMPTION OF KILLER AND SENSITIVE PARAMECIA

Determinations were made using twenty paramecia per diver in respiring medium III. Acetate was added in a final concentration of 10^{-2} and 10^{-3} M at the start of the experimental period. Readings were made immediately before and after the addition of acetate and at hourly intervals for five hours thereafter. The Q_{O_2} values are expressed as millimicroliters of oxygen consumed per animal-hour. For each rate determined in the presence of acetate, a corresponding control rate was obtained at the same time.

Stock	Q_{O_2}		
	Control	*Acetate* ($10^{-2}M$)	*Acetate* ($10^{-3}M$)
51.7 killer	0.23	0.29	0.32
	0.23	0.26	0.27
	0.22	0.22	0.25
	0.19	0.23	0.22
	Mean 0.22	0.25	0.26
51.7 sensitive	0.20	0.36	—
	0.16	0.24	0.21
	0.15	—	0.23
	Mean 0.17	0.30	0.22
d 186.7 sensitive	0.12	0.14	—
	0.12	0.19	—
	0.09	—	0.11
	0.13	—	0.16
	Mean 0.12	0.16	0.14

An increase in respiratory rate of the ciliate, *Tetrahymena geleii* (S), in the presence of glucose and acetate has been reported[21,22]. Also, the respiration of *P. caudatum* is stimulated by about 20% following the addition of glucose to the respiring medium[23].

The fact that the rate of oxygen consumption of the killers was not affected by glucose and was only slightly affected by the addition of acetate could indicate that the killers have a less active system for metabolizing carbohydrate than the sensitives. Another possible interpretation of these results could be made in view of the higher normal respiration of the killers. Certain enzymes of the tricarboxylic acid cycle have been demonstrated in *P. caudatum*[24,25], as well as in *Tetrahymena*[26]. It is therefore likely that a similar system operates in the metabolic scheme of *P. aurelia*. If the tricarboxylic acid cycle in the killers were functioning at near its peak capacity, further addition of substrate would have little effect upon the respiratory rate. The stimulation of the Q_{O_2} of the sensitives then would mean that the tricarboxylic acid cycle in the sensitives is operating endogenously at somewhat less than its maximal capacity. This viewpoint is substantiated by the fact that the highest stimulated respiration of the sensitives approaches that of the killers.

Streptomycin. The Q_{O_2} of the 51.7 killers was stimulated in the presence of streptomycin while that of the d186.7 sensitives was inhibited (Table V).

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TABLE V

THE EFFECT OF ADDED STREPTOMYCIN UPON THE OXYGEN CONSUMPTION OF KILLER AND SENSITIVE PARAMECIA

Streptomycin (5 mg/ml = 3250 units/ml final concentration) was added to the paramecia respiring in medium I. Readings were taken at half-hour intervals for a period of four hours before and four hours after the addition of streptomycin. The Q_{O_2} is expressed in millimicroliters of oxygen consumed per animal-hour. "Percent of Control" is the ratio of the Q_{O_2} values before and after addition of streptomycin. The values in parenthesis represent the ratio of the mean Q_{O_2} values.

Stock	*No. of Animals*	Q_{O_2} *Before Addition*	Q_{O_2} *After Addition*	*Percent of Control*
51.7 Killer	16	0.66	0.80	121%
	16	0.69	0.69	100
	13	0.69	1.02	148
	Mean	0.68	0.84	123% (124%)
d 186.7 Sensitive	12	0.39	0.31	80%
	14	0.25	0.19	76
	16	0.34	0.15	44
	Mean	0.33	0.22	67% (66%)

Streptomycin has been demonstrated to be effective in the blocking of one of the reactions involved in the condensation of pyruvate or acetate with oxalacetate in the tricarboxylic acid cycle[27,28,29]. The inhibition of the respiration of the sensitive paramecia by streptomycin indicates that the tricarboxylic acid cycle or a similar cycle operates in the sensitives. The stimulation of respiration of the killers in the presence of streptomycin suggests that in the killers some additional oxidative system may act independently of the tricarboxylic acid cycle. The stimulation of the respiration in the killers could result from the blocking of the tricarboxylic acid cycle thus supplying extra substrate for the alternative system.

Sodium azide. The results of respiratory studies on killer and sensitive animals in the presence of two different concentrations of azide ($2.5 \cdot 10^{-3}$ and $1 \cdot 10^{-4}$ M) are reported in Table VI. At both concentrations of azide the Q_{O_2} of the kk sensitive animals is intermediate between that of the KK killers and sensitives. The killers in the presence of azide respire at the highest rate and the KK sensitives at the lowest. The fact that the respiratory rate of the killers was stimulated at an azide concentration of 10^{-4} M while that of the KK sensitives was inhibited led to a further investigation in which a range of azide concentrations from 10^{-2} to 10^{-7} M was used (Fig. 2). The results of these experiments are similar to those obtained from the first series, and there was the same difference in effect among the three types of paramecia studied. Within the concentration range of 10^{-3} to 10^{-5} M, azide, the respiration of the killer animals was stimulated while that of the corresponding KK sensitives was inhibited. The respiration of the kk sensitive stock showed an intermediate response with a slight stimulation over its control respiration.

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A further difference of the effect of azide upon these stocks was noted in the length of time during which the animals would remain active and continue to respire in the presence of azide (Table VII). Again, the KK sensitive paramecia were most sensitive to azide while the killers were insensitive.

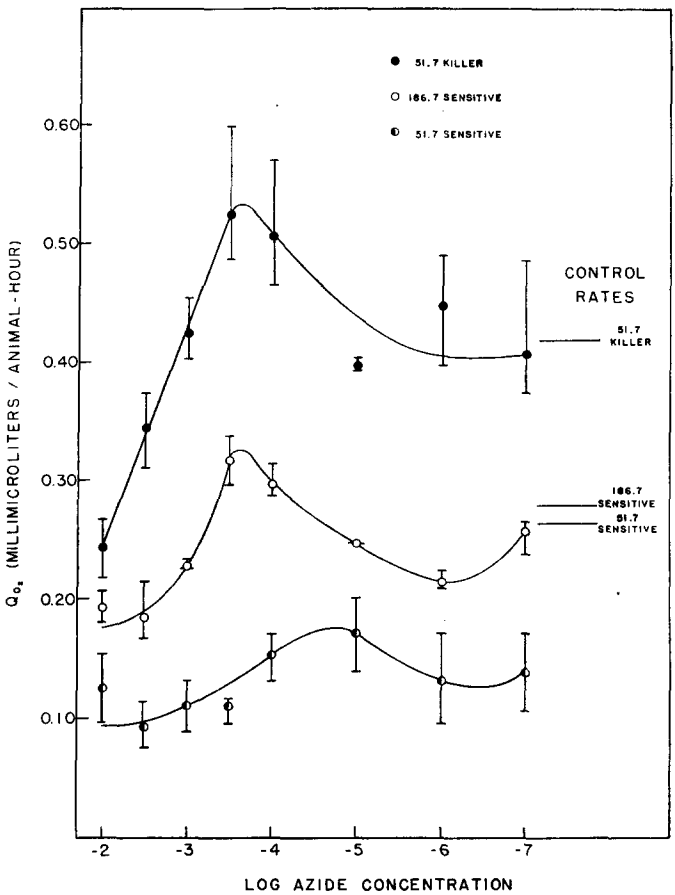

Fig. 2. Effects of different concentrations of sodium azide upon the rates of oxygen consumption of killer and sensitive paramecia. Rates were calculated for the period of survival of the animals in the diver (*cf.* Table VII). Determinations were made using twenty paramecia in each diver. The individual points on the curves represent four determinations. The vertical lines indicate the range.

The azide sensitivity of the 51.7 KK sensitive animals indicates that an iron containing system, the cytochrome system, must act as the principal mechanism of respiration. The existence of this system in other species of Paramecium[24,25,30,31] as well as in *P. aurelia*[23] has been established. The fact that the same concentrations of azide which inhibited the respiration of the sensitive animals stimulated the respiration of the killers indicates that an important auxiliary oxidative system, different from the usual cytochrome system in its sensitivity to azide operates in the respiratory processes of the killers. This alternative system must then be the result of the presence of kappa in the cell, or could even be directly associated with the kappa particle. The differences

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between the two stocks of sensitives of different genotype (*i.e.* the stock d186.7, kk, and the 51.7, KK) with respect to their response to azide suggests that there is a different respiratory pattern in the two stocks which, although not apparent from the gross respiratory studies, is revealed in the presence of azide.

TABLE VI

THE EFFECT OF ADDED SODIUM AZIDE UPON THE RATE OF OXYGEN CONSUMPTION OF KILLER AND SENSITIVE PARAMECIA

Sodium azide solutions were made up fresh, daily, and the pH was adjusted to 7.0. The experiments were run using twenty paramecia in each diver. Readings were made immediately before and after addition of azide and at hourly intervals for five hours thereafter. In the higher concentration some of the animals did not survive over the entire five hour period (*cf.* Table VII). In these experiments the rate was calculated for the period of survival. The Q_{O_2} is expressed as millimicroliters of oxygen consumed per animal-hour.

Stock *Genotype* *Phenotype*	51.7 *KK (x)* *Killer*		51.7 *KK* *Sensitive*		d 186.7 *kk* *Sensitive*	
	Q_{O_2}		Q_{O_2}		Q_{O_2}	
	No. of Expts.	*Means* $\pm$ *S.E.*	*No. of Expts.*	*Means* $\pm$ *S.E.*	*No. of Expts.*	*Mean* $\pm$ *S.E.*
Control	9	0.36 ± 0.03	7	0.22 ± 0.03	7	0.23 ± 0.03
Azide ($2.5 \cdot 10^{-3}$ *M*)	9	0.32 ± 0.03	7	0.06 ± 0.02	7	0.14 ± 0.02
% of Control		89 %		29 %		60 %
Control	8	0.39 ± 0.03	8	0.26 ± 0.02	7	0.22 ± 0.03
Azide ($1 \cdot 10^{-4}$ *M*)	8	0.53 ± 0.04	8	0.16 ± 0.02	7	0.22 ± 0.04
% of Control		136 %		62 %		102 %

TABLE VII

HOURS OF EXPOSURE TO VARYING CONCENTRATIONS OF AZIDE BEFORE CESSATION OF MOVEMENT AND RESPIRATORY ACTIVITY OF KILLER AND SENSITIVE PARAMECIA

Stock *Genotype* *Phenotype*	51.7 *KK (x)* *Killer*	51.7 *KK* *Sensitive*	d 186.7 *kk* *Sensitive*
Molar Azide Concentration	*Time (hours)*	*Time (hours)*	*Time (hours)*
$10^{-2.0}$	2	1	2
$10^{-2.5}$	—	2	3
$10^{-3.0}$	—	3	4
$10^{-3.5}$	—	—	—
$10^{-4.0}$	—	—	—

— indicates that the paramecia were still living at the end of the five hour experimental period.

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Details of the curves in Fig. 2 are more difficult to explain. If the inhibition involved only the cytochrome oxidase system, higher respiratory rates with decreasing concentrations of azide would be expected. However, in each of the curves a maximum is noted which in the d186.7 sensitives and particularly in the killers exceeds the normal endogenous respiratory rate. The initial rise in rate with decreasing concentration is probably due to the release of inhibition in the cytochrome oxidase system. This would not account for the stimulation above the normal respiratory level or the second inhibitory phase, and some other azide sensitive system must be involved. Azide is known to act in uncoupling phosphorylation from oxidation, thereby inhibiting synthetic mechanisms at concentrations which are not completely inhibitory to respiratory processes[32,33]. A block in the synthetic mechanisms should release more substrate to be broken down by the oxidative processes. The maxima in the curves then would represent the lowest concentration of azide at which the synthetic processes are completely inhibited. Below this concentration, the extra substrate would be diverted back into synthetic channels and the level of oxidation would approach the normal level. The fact that the respiration of the KK sensitives is still more strongly inhibited at concentrations below 10^{-5} *M* further indicates the presence of the cytochrome system as the main oxidative system in this stock.

The higher peak of stimulation in the killers might be expected because the synthesis of kappa and paramecin must draw heavily upon the energy reservoir of the killer animals. A block of the synthesis of kappa and paramecin would release more substrate to be oxidized in the killers than in the sensitive animals. Further, the alternative azide-resistant oxidative system, associated with the presence of the kappa particle, would be immediately available to take over the oxidation of the extra substrate.

The intermediate reaction to azide shown by animals of the d186.7, kk sensitive stock indicates that the presence of the k gene or the absence of the K gene may be influencing the pattern of azide sensitivity. However, the possibility cannot be ruled out that some other residual gene or genes from stock 29 which control this pattern, may have persisted through the seven backcrosses.

Cytochrome oxidase. The results of the cytochrome oxidase determinations are presented in Table VIII. In these experiments, the "total oxidase" activity of the killers (*i.e.* the rate of oxidation of the dye in the presence of excess cytochrome C) was about 65% of that of the sensitives of either genotype. However, in the absence of added cytochrome C the breis of all three types of animals were able to oxidize the dye at an appreciable rate. These "dye oxidase" activities for killer and sensitive animals were not significantly different. The net cytochrome oxidase activity (*i.e.* the difference between the rates of oxidation of the dye in the presence and absence of added cytochrome C) of the killers is about 35% that of the sensitives.

The nature of the dye oxidase activity is unknown[19,34], but it may be a measure of the availability of cellular cytochrome C in these determinations. The total oxidase activity would then be the true measure of the cytochrome oxidase activity. If, however, the oxidation of the dye were carried out by some other oxidative system, the difference between the total oxidase and the dye oxidase activities would represent the cytochrome oxidase activity. Thus, depending upon the interpretation of the dye oxidase, the cytochrome oxidase activity of the killers would be either 35% or 65% that of the sensitives. Cyanide in a concentration of $5 \cdot 10^{-3}$ *M* completely blocked all oxidase activity.

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TABLE VIII

CYTOCHROME OXIDASE ACTIVITY OF BREIS OF KILLER AND SENSITIVE PARAMECIA

Determinations were made using breis corresponding to a concentration of 25,000 to 30,000 paramecia per ml. The oxidase activity is expressed in millimicroliters of oxygen consumed per animal-hour.

Stock No.	51.7		51.7		d 186.7	
Genotype	*KK (x)*		*KK*		*kk*	
Phenotype	*Killer*		*Sensitive*		*Sensitive*	
Substrate	Q_{O_2}		Q_{O_2}		Q_{O_2}	
	No. of Expts.	*Mean ± S.E.*	*No. of Expts.*	*Mean ± S.E.*	*No. of Expts.*	*Mean ± S.E.*
Brei + Dye + Cytochrome C	10	0.102 ± 0.006	9	0.155 ± 0.010	9	0.159 ± 0.012
Brei + Dye		0.076 ± 0.006		0.086 ± 0.004		0.084 ± 0.003
Net Cytochrome Oxidase Activity		0.026		0.069		0.075

A consideration of these oxidase values in relation to the normal endogenous respiratory rates in the same medium (*i.e.* Medium III) provides further proof for the existence of an alternative system of oxidation in the killers. The total oxidase activity of the sensitives of either genotype was 0.16 which agrees closely with their endogenous respiratory rates (*i.e.* 0.16 for the KK genotype and 0.13 for the kk genotype). The oxidase activity of the killer was 0.10 as compared to a respiratory rate of 0.24. It is apparent that this oxidase activity is not nearly high enough to account for the endogenous respiratory rate. Thus the killer must carry out oxidative processes *via* some different oxidative system in addition to the cytochrome system.

DISCUSSION

The differences in respiratory rates and the effects of added acetate and glucose indicate that the respiratory processes in the killers *via* the tricarboxylic acid cycle are carried out at their maximal rates. This might be expected if a high level of synthetic activity were necessary for the maintenance of the kappa level and the continual production of paramecin. The effect of streptomycin is interpreted to mean that, in the killers, the tricarboxylic acid cycle is not the only pathway for the oxidation of substrate. The relative azide insensitivity of the killers and the lowered cytochrome oxidase activity indicate that the normal cytochrome system is not the principal oxidative system in the killers. The nature of the alternative system is unknown, but it may be of the same type as the cyanide and azide insensitive systems postulated by other workers—possibly a flavin system[35, 36, 37, 38].

The results of these respiratory studies, coupled with the observation that kappa is a particulate cytoplasmic component, suggest that kappa may play an integral part in the enzymic organization of the killers. The bulk of evidence which has been

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gathered by means of cell fractionation techniques has pointed to the important role of particulate structures in the metabolism of the cell[39,40]. A multiplicity of enzymic processes has been found to be associated with the mitochondria. A fact which is of particular interest in connection with this study, is that cytochrome oxidase in all of the tissues studied was associated exclusively with the mitochondrial fraction[39]. Therefore, the observation that the killers have a lowered cytochrome oxidase activity indicates that there may be fewer mitochondria in the killers than in the sensitives. This would imply that in the presence of kappa the synthesis of the mitochondria is inhibited. If these two systems are mutually antagonistic because of competition with each other for substrate, a formal scheme can be postulated as shown in Fig. 3.

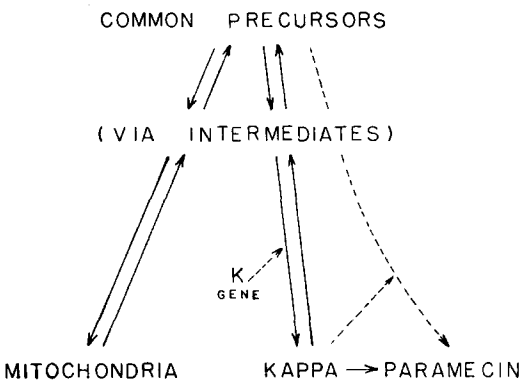

Fig. 3. Proposed scheme of interrelationship between kappa and mitochondria.

Since kappa produces or controls the production of paramecin, which in turn is removed from the site of synthesis by discharge into the culture medium, an equilibrium could be established which would favor the pathway to kappa synthesis, and the synthesis of the mitochondria would be inhibited. The gene K, could act by controlling the presence of any one of the enzymes which must act in the production of kappa. For example, it might act in the conversion of mitochondrial ribosenucleic acid to desoxyribosenucleic acid which is a principal component of both kappa and paramecin. The mutability of kappa in the absence of a mutation in the K gene would rule out the possibility that the K gene is acting by conferring a complete specificty upon kappa.

Thus, kappa, containing a complex of respiratory enzymes, would have a similar function as the mitochondria. EPHRUSSI[41,42] has demonstrated in yeast the cytoplasmic inheritance of a respiratory system containing cytochrome oxidase. This system is different from the kappa system with regard to the enzyme system involved, but the fact that he has demonstrated the inheritance of a respiratory system via the cytoplasm, emphasizes the importance of cytoplasmic inheritance in determining the enzymic make-up of the cell.

SUMMARY

An oxidative system different from the normal cytochrome system operates in the respiratory metabolism of killer paramecia. This system is associated with the presence of the cytoplasmic particle, kappa. The higher Q_{O_2} of the killers as compared with the sensitives, the lack of respiratory inhibition by sodium azide in concentrations which inhibit the respiration of the sensitives, as well as the low cytochrome oxidase activity of the killers, support this conclusion. Differences between killers and sensitives in their respiratory response to acetate, glucose, and streptomycin provide further evidence of an altered metabolic pattern in the killers.

RÉSUMÉ

Une système oxidative, différente de la système cytochrome normale, est opérative dans le métabolisme respiratoire des paramécies "killer". Cette système est associée avec la présence de la particule cytoplasmique "kappa". Le Q_{O_2} èlevé des paramécies "killer", l'absence de l'action d'inhibiteur, l'azoture de sodium (en concentrations qui inhibent la respiration des paramécies "sensi-

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tives"), et l'activité faible de la cytochrome oxidase des paramécies "killer" supportent cette conclusion. La différence entre les paramécies "killer" et les paramécies "sensitives" dans leur réponse respiratoire à l'acétate, à la glucose, et a la streptomycine constitute évidence additionelle d'une mètabolisme altéré dans les paramécies "killer".

ZUSAMMENFASSUNG

Der aerobe Stoffwechsel von "Killer"-Parämazien erfolgt durch ein Oxydations-System, das vom normalen Zytochrom-System verschieden ist, und das an die Gegenwart von "Kappa"-Teilchen im Zytoplasma gebunden ist. Diese Anschauung stützt sich auf den höheren Q_{O_2}-wert der "Killer" im Vergleich zu "Sensitive"-Paramäzien, auf das Ausbleiben einer Hemmung der Atmung durch Natrium-Azid in Konzentrationen, welche die Atmung von "Sensitive"-Paramäzien hemmen, und auf die geringe Zytochrom-Oxydase Aktivität in "Killer"-Paramäzien. "Killer" und "Sensitive"-Paramäzien reagieren auch verschieden auf Zusatz von Acetat, Glukose oder Streptomyzin; dies ist ein weiterer Beweis für Unterschiede in ihrem Atmungstypus.

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