STUDIES ON KAPPA-LIKE PARTICLES IN SENSITIVES OF PARAMECIUM AURELIA, VARIETY 4 [1]

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KILLER paramecia are resistant to the effects of the poison, paramecin, which they produce. Paramecia susceptible to this same poison are called sensitives (SONNEBORN 1939, 1943). The killer character in *Paramecium aurelia* depends on the presence both of the *K* gene in the nucleus and of the self-reproducing, mutable, microscopically visible particles, called kappa, in the cytoplasm, whose presence is correlated with the production of paramecin (PREER 1946, 1948b; SONNEBORN 1946). The replacement of the *K* gene of killers by its recessive allele, *k*, causes the disappearance of kappa from the cytoplasm with a concomitant loss of killing ability, which results, finally, in sensitive lines which do not possess kappa (SONNEBORN 1943). The loss of kappa from a variety 4 killer, whether due to genetic, as just mentioned, or environmental reasons, results in the transformation of that animal into a sensitive whose progeny are all sensitive (WILLIAMSON, JACOBSON and STOCK 1952; PREER 1948a; SONNEBORN 1946; VAN WAGTENDONK, CONNOR and MILLER 1953).

The sensitive, non-killer character thus results from the absence of kappa. It is also known to occur when the number of kappa particles per cell is low (SONNEBORN 1946). According to estimates by SONNEBORN (1946), PREER (1948a), and CHAO (unpublished) the cells are sensitive if there are less than about 20 kappa particles in it. The present paper reports a new type of sensitive which arose unexpectedly from killer ancestors. Curiously, it has the genotype and seems to have the cytoplasmic condition proper to a killer, i.e., it carries gene *K* and large numbers of cytoplasmic kappa-like particles. Such an unprecedented constitution for a sensitive obviously leads to important modifications of the present views of the killer-sensitive system. These will be discussed after presenting an account of the origin and genetic analysis of this sensitive and a preliminary report on the behavior of kappa and the cytoplasmic particles of the new sensitive when both are in a common cytoplasm.

MATERIALS AND METHODS

Animals of *Paramecium aurelia*, varieties 2, 4 and 8, were used in the work reported here. The variety 4 animals are: stock 51, the standard killer stock

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(SONNEBORN 1943); stock d186, a derived stock containing the *k* gene from stock 29 but otherwise essentially isogenic with stock 51 (DIPPELL 1950); stock 47, a killer; and stock 139, isolated as a killer from nature but now a sensitive. All of these variety 4 stocks are interfertile giving viable F_1 and F_2 progeny. Stocks of variety 2 were used only as killer testers. Stock 31, variety 8, was used only as a standard sensitive tester. The killing action of stock 51 (DIPPELL 1950; SONNEBORN 1943), stock 47 (DIPPELL 1950), and the variety 2 killers (PREER 1948a) has already been described.

The methods used for maintaining the cultures, making the crosses, inducing autogamy, setting killer tests, and obtaining specific antisera are those set forth by SONNEBORN (1950).

ORIGIN, DESCRIPTION AND GENETIC ANALYSIS OF THE SENSITIVES

Origin. Normally the F_2 derived by autogamy from the cross between two homozygous killer parents are all killers. However, in one such experiment, the F_2 consisted of two sensitives and 122 killer clones. In this experiment both parents were killers homozygous for gene *K*. One parent was stock 51,

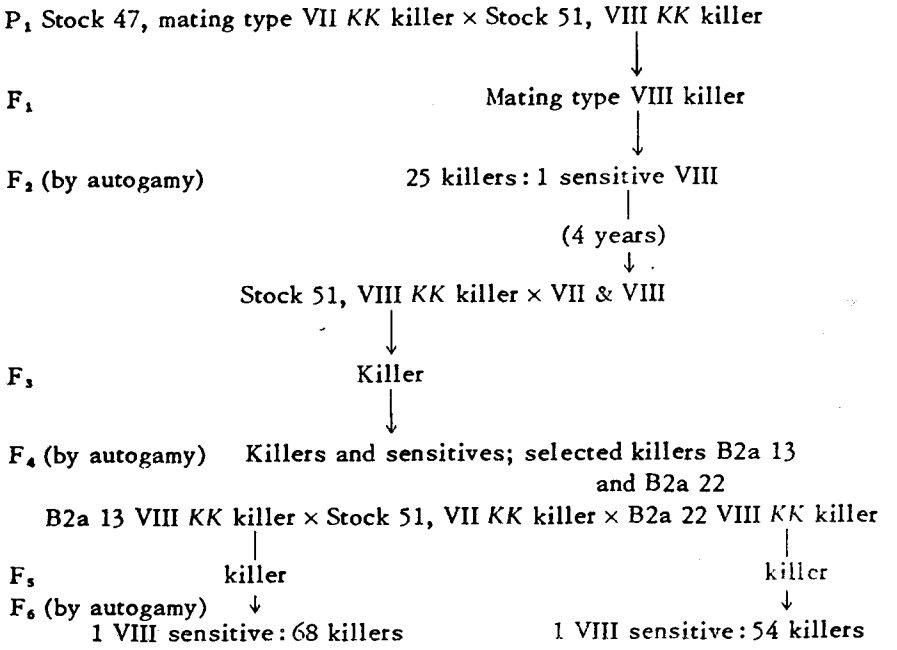

FIGURE 1.—Diagram showing genetic history of the two exceptional sensitives.

mating type VII, and the other was mating type VIII but of hybrid origin, as set forth in figure 1. The two killers B2a 13 and B2a 22, which were taken from the F_4, are the ones used for the cross to stock 51. It was in the F_6 that the exceptional sensitives were found. Another unexpected sensitive was the one found in the F_2. The analysis of this line will be reported elsewhere and

is thought not to have any bearing on the present case for reasons to be brought out later.

Phenotype. The diagnosis of these two exceptional cultures as sensitives is based on two facts: they are killed when mixed with stock 51 killers or with breis of these killers; and they and their breis cannot kill standard sensitive cultures.

The action of killers other than stock 51 killers has also been tested on these animals. The reason for testing these other killers was that DIPPELL (1950) has found mutations of stock 51 kappa which result in the mutant killers being resistant to their own paramecin but not to that of the original 51 animals. It was conceivable that in the special sensitives being considered here, there had been two mutations; one which changed the type of killing action and therefore also the type of resistance, and then a second which eliminated the ability to kill. The final result being animals unable to kill stock 51 and at the same time being sensitive to it, but retaining, perhaps, a resistance to some other kind of paramecin. However, in no case was any resistance detected. These sensitive lines are susceptible to the paramecin produced by the following killers: stock 51 and stocks G, 36 and 308-2 of variety 2. It was found that the sensitive lines were killed in the manner typical for each type of killer.

Genotype. A priori it was conceivable that these lines had become sensitive to the action of paramecin due to a nuclear change, e.g., mutation of the *K* gene to its recessive allele, *k*, which cannot support kappa. One such case is already known (HANSON unpublished). This is the exceptional case mentioned earlier and represented by the sensitive found in the F_2 (fig. 1). Breeding analyses were carried out to test this hypothesis in the present case.

Each sensitive was crossed to a known standard pure killer. Stock 51 was used as this killer. If the sensitive parent lacked gene *K* or any other gene necessary for the maintenance or expression of the killer trait, the F_1 should be heterozygous at this locus and the F_2 obtained by autogamy should segregate killers and sensitives in a 1 : 1 ratio. On this assumption the crosses were followed through to the F_2. The results, summarized in table 1, show that no segregation occurred in the F_2; all of the F_2 were killers and of the standard stock 51 type. The marked difference in results when a stock with a known

TABLE 1

Summary of the results of crossing the sensitives of anomalous origin to stock 51. (Symbols: (K) = killer, (S) = sensitive)

Cross	Number of F_2 clones obtained	Results					
		Observed		Theoretical			
				Single gene difference		No gene difference	
		(K)	(S)	(K)	(S)	(K)	(S)
Sensitive line A × Stock 51	120	120	0	60	60	120	0
Sensitive line B × Stock 51	95	95	0	47.5	47.5	95	0
Stock d186 × Stock 51	116	60	56	58	58	116	0

single gene difference (*kk* instead of *KK*) is similarly crossed to stock 51 is also shown in table 1; here a 1 : 1 segregation of killers and sensitives does occur in the F_2. This cross serves as a control on the experimental cross. Hence, it may be concluded that the sensitives do not differ from the standard killers in any gene affecting the killer trait.

Cytoplasm. With the elimination of the possibility of a relevant nuclear mutation in the origin of the sensitive clones, attention was directed to the cytoplasm. As a working hypothesis it was assumed that kappa had, for undetermined reasons, been lost from these animals. This hypothesis was tested by staining the lines in question using DIPPELL and CHAO's modification of DELAMATER's basic fuchsin technique (SONNEBORN 1950), and examining them for the presence or absence of kappa particles.

The results of this cytological study were quite unexpected: kappa-like particles were found in the cytoplasm of these animals. A preliminary estimate would place the number of these particles as about 400 per cell.

Using the techniques of phase microscopy developed by PREER and STARK (1953) it has been found that the shape of these particles differs from that of kappa. According to PREER and STARK (1953), kappa in variety 4 consists of essentially two types of particles: (1) subspherical or ovoid bodies averaging 1.2 micra in length, either single or, less commonly, double—a long "single" with a median constriction; (2) larger and more irregularly shaped particles which may or may not contain a refractile body. The particles found in the sensitives do not show this dimorphism. These particles are invariably rod-shaped; they appear as singles, doubles, even triples, and, rarely, quadruples. No refractile bodies have ever been observed in them. In lines fed to multiply at 0.5 fissions per day, the size of single particles in a sample of 30 varied from 1.1 micra to 5.4 micra, with a mean value of 2.7 micra. The compound forms may be as large as 10 micra. These observations were made under bright phase (American Optical bright H objective) at a magnification of 1455×.

PREER and STARK (1953) have reported the possibility of serological studies on kappa in breis made from killers. In making these studies whole animals are broken up on a glass slide in a drop of the undiluted testing serum and then observed under bright phase. Using this method it has been possible to demonstrate a serological difference between kappa and the particles in the sensitives. Using antisera prepared against stock 51 killers, against stock 29 which contains no kappa-like particles, and against the particle-containing sensitives, it was found that kappa was agglutinated only in the presence of the anti-killer serum, and the new particles only in the presence of their specific antiserum. This agglutination takes place rapidly: in a matter of minutes the particles stick to each other in a random manner, regardless of whether they are singles, doubles or of larger size.

From the observations reported above it is clear that kappa and these newly found particles differ in a number of respects, such as size, shape, serologic properties, and paramecin production. Because of these differences the new particles have been provisionally designated "pi" in contradistinction to the

already familiar kappa. The implications of this designation are meant to be twofold. (1) Because of the differences just cited, the origin of pi as a mutant kappa may be doubted, a point that will be discussed more fully later. Therefore the terminology used by DIPPELL (1950) for kappa mutants has not been used. (2) These pi particles are unique in two respects in the killer-sensitive system of *P. aurelia*. First, the high concentration of the particles in each animal eliminates the possibility that these animals are sensitives as a result of a low kappa concentration (SONNEBORN 1946). Second, the lack of any detected killing ability and the lack of resistance to any killer, differentiates pi sensitives from DIPPELL's 51m5 mutant which showed little or no killing ability but yet was resistant to stock 51 paramecin.

Relation of pi to the genetic constitution of the paramecia. An experiment was designed and carried out to test the possibility that pi, like kappa, is dependent on the *K* gene for its continued survival. The test was to cross the pi-bearing sensitives to *kk* sensitives, to obtain autogamy in the pi-bearing F_1, and to ascertain whether the F_2 generation thus obtained segregates 1 : 1 for the capacity to maintain pi. If so, then each F_2 culture would have to be analyzed by crosses to discover whether the cultures that lost pi are homozygous for gene *k*, while those that retained pi are homozygous for gene *K*.

The details of this analysis are as follows: The *kk* sensitive stock employed was d186. It was crossed to a pi-bearing sensitive (*KK*), marked phenotypically by a different serotype from that manifested by d186. The pi-bearing F_1, as determined by its serotype and confirmed in two cases by staining for pi, was used to obtain an F_2 generation by autogamy. Each of 47 cultures was (1) stained to ascertain whether pi was present or not; and (2) crossed to pure killers (*KK*) of stock 51 to determine by the character of the F_2 obtained from the killer F_1, whether the tested cultures carried *K* or *k*. In this second test, as in the experiments set forth above (page 233), each tested culture that carried gene *K* would yield only killers in the F_2. The data of the entire analysis are summarized in table 2, which shows that pi, like kappa, is maintained only in the presence of the *K* gene. All 22 of the F_2 cultures shown to carry *K* also carried pi; all 25 shown to carry *k* lacked pi.

The only observation which interferes with the decisiveness of the experiment is that in three of the lines homozygous for the *k* gene, staining by the basic fuchsin technique revealed a small number of animals containing particles similar in size and number to pi. The reason for the presence of these particles is obscure. They are in all probability not due to the slower loss of particles in these lines as compared to their sister lines because (1) the concentration appears to be essentially normal, and (2) because, using kappa loss (CHAO 1953) as a parallel, the particles should not have persisted beyond the 14th fission after the animals became homozygous for gene *k*, and these lines were past their 40th fission. A possible explanation is that a few of the descendants of a single autogamous isolation regenerated their macronucleus from a fragment of the previous macronucleus (SONNEBORN 1945a, 1945b). The regenerated macronucleus would of course, in these crosses, be heterozygous at the *K*

TABLE 2

Summary of the results from the genetic and cytological analysis of a pi containing culture to determine whether or not pi needs the K gene for its continued presence in an animal.

Cross	Total number of F_2 clones	Results (K)	Results (S)
Experimental:			
Pi sensitive × Stock d186	54	0	54
Controls:			
Pi sensitive × Stock 51 killer	137	137	0
Stock d186 × Stock 51 killer	127	67	60

Of the 54 lines of sensitives from the experimental cross above, 47 were analyzed genetically for the presence of the K or k gene, and cytologically for the presence or absence of pi.

	Genetic results	
	Number of crosses showing 1 : 1 segregation in F_2	Number of crosses showing no segregation in F_2
Each sensitive clone crossed to Stock 51 killer	25	22
	Cytological results	
	Lines not showing pi	Lines showing pi
Same sensitive clones	25	22

In each of the lines carrying the K gene pi was also found; in each of the lines carrying the k gene no pi was found. (See text for temporary exceptions.)

locus and could still maintain pi in the paramecium in spite of the presence of a *kk* micronucleus (SONNEBORN 1946b). Since no killing would occur in these cultures, animals with and without pi would both survive. Pi would, however, be lost whenever an animal containing it would undergo a normal autogamous nuclear reorganization, as this would cause the macronucleus containing the *K* gene to be replaced by a new macronucleus not containing it. In line with this interpretation is the fact that a considerably later repetition of the staining, using PREER's (unpublished) crystal violet technique was used on these same lines and no evidence of particles was found.

Mixed cytoplasms. Of especial interest is the question of what happens when cytoplasm is exchanged during conjugation (SONNEBORN 1950) between pi and kappa containing animals. Repeated observations show that both exconjugants produce killer cultures by the eighth post-conjugational fission, the culture from the former sensitive member of the pair gradually transforming into a killer, and the culture from the killer member simply maintaining its original killer character. Furthermore, if these exconjugant clones are carried along by continual subculturing either by daily isolation of single animals or of small random samples, dead animals are found in the cultures when they

are allowed to starve down. No death has been observed in the well-fed cultures. The amount of death varies from clone to clone. The death appears to be due to the action of paramecin since the corpses have the appearance of well-starved sensitives that have been exposed to stock 51 paramecin.

One explanation for the origin of these " sensitives " was thought to lie in the fact that since pi was known to have replaced kappa once (when pi was first found), that it therefore was conceivably doing it again with the result that the sensitives were being killed off, as fast as they appeared, by the remaining killers in the culture. To test this possibility numerous isolations were made both before and after the mortality set in, with the intent of isolating, before it was killed, an animal that was to become a sensitive. None of these isolations ever established pure sensitive lines, though they did continue to throw dead animals. Even repeated selection from the cultures showing the greatest amount of this autolethality did not yield a single sensitive culture.

Stock 139. Stock 139 when first brought into the laboratory from nature was a killer. All the cultures of this stock which are now on hand are sensitives. Cytologic examination of this stock shows that pi-like particles are also present. The exact history of the change from killer to sensitive is not known for these animals.

DISCUSSION

Origin of pi

Two explanations of the origin of pi need to be considered: that pi is a mutant kappa; and that pi represents an independent infection from an exogenous source.

Mutation of kappa. DIPPELL (1950) has already reported that stock 51 kappa can mutate. However, the mutations analyzed by DIPPELL were in all cases changes in the killing character of the mutants, i.e., the original " hump " type killers became either " spinner " killers or very weak hump killers that were still resistant to their own type of paramecin. PREER (1948a) also described a variety 2 mutant, Gm1, which shows a different type of killing than that manifested by the original stock G killer. Mutations from killer to sensitive could be detected only under certain conditions. The sensitives would have to be isolated from the killer culture before they were affected by the killers, for otherwise they would be lost. But they must not be isolated so early that the resulting culture would produce, by a chance segregation at fission of killer and non-killer particles to the daughter cells, any paramecia that contained only the non-mutated particles, as this would reestablish a pure killer line. It will be recalled that these conditions could have been realized when the pi-bearing lines were first discovered because both pi lines were isolates from killer cultures. From these considerations it is not unreasonable to assume, *a priori,* that paramecin-producing kappa might mutate to a non-paramecin-producing form which could be detected if the unmutated kappa were replaced by the mutated kappa, and if the animal containing it were isolated from the killer culture at the appropriate time.

Three lines of evidence supporting this mutation hypothesis can be offered. First, the pi lines were derived from kappa-bearing lines. Second, pi, like kappa, needs the *K* gene for its continued maintenance. Third, pi and kappa are demonstrable by the same cytological techniques.

There are, however, certain facts which are not readily reconciled with the mutation hypothesis, though they are not fatal to it. From the description of pi it can be seen that there are certain points of dissimilarity between these particles and kappa. Pi does not produce paramecin, animals possessing pi are not resistant to any tested paramecin, pi is morphologically and serologically distinguishable from kappa, and, lastly, there is some difference, as yet unknown, which determines an apparent mutual exclusion effect when pi and kappa are in the same animal, such that in one instance (that of the original discovery of pi) kappa was replaced by pi, but in other instances, after cytoplasmic exchange occurs between pi- and kappa-bearing animals during conjugation, only killer animals have been recovered. These multiple differences can be explained by any one of three different postulates. (1) The kappa mutation that gave rise to pi is pleiotropic in its effect; (2) there has been an accumulation of different mutations in the particles, including one that eliminated the killing ability and resistance of the animals; and (3) a combination of points (1) and (2). To distinguish clearly between these alternatives will be difficult. However, on the basis of the second and third postulates, one would expect that, if enough different characteristics of the particles were studied, differences would be detected between particles of different origin due to the fact that unlike mutations have probably accumulated in the different lines. If no differences are found the first alternative would be indicated. Preliminary comparison of pi particles in stock 139 with those in the lines reported here show no significant differences.

Infection from an exogenous source. The reasons for entertaining this hypothesis as a possible explanation for the origin of pi are: if kappa is a " viroid " or a relative of the green alga of *P. bursaria* as ALTENBURG (1946) suggests, or if kappa can be compared with Rickettsiae as PREER (1950) has suggested, then pi, also, could be of extrinsic origin and from a source different than kappa.

The critical investigation is to determine whether pi appears only in lines which carry kappa. In the cases known thus far pi has been found only under such conditions. This is true not only for the two lines analyzed in this paper, but also for stock 139, and for certain lines examined by PREER, SIEGEL, and STARK (1953). The latter have found pi-like particles in certain of the mutant killers of stock 51 earlier analyzed by DIPPELL (1950). Yet to be discovered is whether pi ever exists in lines known not to have contained kappa. In the light of our present knowledge, therefore, the mutation hypothesis is thought to be the more likely explanation for the origin of pi.

Pi and kappa in a common cytoplasm

If it can be assumed that there is no intracytoplasmic selection for either pi or kappa, that on the average they both multiply at the same rate and that new

origins of pi are so rare as to be statistically negligible—in sum then, that random segregation is the only factor governing the distribution of these particles, then the replacement of kappa by pi on this basis would be a rare but possible event. However, there are two points which indicate that random segregation is not the only factor involved here.

First, in a cross such as described in the mixed cytoplasm work, two different situations conceivably exist in the respective exconjugants of a single pair. At the start the killer member of the pair possesses an excess of kappa over pi in its cytoplasm, and in the sensitive member the reverse holds true. The probability of chance segregation alone yielding any pure pi sensitives (lacking kappa) from the killer clone would be low, just how low depending inversely on the amount of cytoplasm exchanged. And conversely, in the clone derived from the sensitive parent, the probability of getting cytoplasms pure for kappa on this same basis would be similarly low. Which is to say, if we isolate the members of the clone derived from the sensitive and avoid selection due to the action of paramecin, we should find a preponderance of sensitive as compared to killer subcultures. But this is not the case. As stated previously, what is found is all killer subcultures. The first point, then, is that random segregation of the two types of particles is not apparent under the conditions described here.

The other point is that in the exconjugant clones corpses are found. The dead animals appear to have died as a result of the action of paramecin, and presumably then, they were sensitives. If these sensitives are due to the replacement of kappa by pi, as has already been suggested, and if this replacement is dependent on random segregation alone, then it should be possible to isolate pi-sensitive lines from these cultures. For example, if in a given culture 10% of the animals were dying, then isolation of 100 animals from this culture at the appropriate time should yield roughly 10 lines of pi-containing sensitives. But as has already been reported, contrary to expectations, no sensitive lines have ever been established. This is also taken to indicate that random segregation of these particles is not solely responsible for their distribution.

In this connection should be mentioned the observation made by DIPPELL (1950) that the normal stock 51 kappa—" hump " kappa—when in the same cytoplasm as either " spinner " or " resistant " kappa (both of these latter are mutations of hump kappa) does not result in the appearance of any dead animals in such cultures. DIPPELL, therefore, concludes that a sort of population equilibrium is set up within such animals as regards the kappa they contain, such that both types persist within a single animal. But it is admitted that this equilibrium concept does not readily explain how both of these mutants were established spontaneously as pure mutant cultures lacking completely the hump kappa.

The nature of the other factors involved in the distribution of pi and kappa is not known. They may be concerned with different rates of multiplication of the two types of particles, which may in turn depend on the competition for a common precursor, e.g., the *K* gene product. Or again, it may simply be due to as yet unidentified factors in the culture technique which may or may not

affect the two possibilities first suggested. These speculations are now under experimental analysis in an attempt to obtain additional information which will throw light on the processes involved in the establishment, maintenance, and transmission of these materials of cytoplasmic heredity.

SUMMARY

A new type of sensitive has been found in the killer-sensitive system of *Paramecium aurelia*. This sensitive contains in its cytoplasm a type of particle not encountered before in this organism. The particles, referred to as pi, are thought to be mutant kappa particles. They arose from a killer culture; they are dependent on the *K* gene for their survival; and they possess the same staining capacities as kappa. Certain points of dissimilarity between pi and kappa are also described and discussed. The present extent of the knowledge of the interrelationships between pi and kappa, when both are in a common cytoplasm, are presented and briefly discussed.

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