

SPONTANEOUS BACTERIAL MUTATIONS TO RESISTANCE TO ANTIBACTERIAL AGENTS

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Bacterial genetics differs from the genetics of higher organisms chiefly because of lack of information about the mechanism of orderly transmission of characters from generation to generation, owing to the absence of well-established processes of conjugation and to the infant state of bacterial cytology. Our only way of approach is the study of bacterial mutability, for which the field of bacterial variation offers a wide range of material. One of the first tasks we face is that of establishing how closely mutations in bacteria are comparable to the so-called gene mutations in higher organisms. Geneticists as well as bacteriologists have often thought that the genetic system of bacteria may be less orderly; in particular, that properties may be transmitted at fission by more or less random distribution of substances present in the protoplasm, without special mechanisms for equational division.

If a case could be made, however, for similarity of the processes of mutation in bacteria and in higher organisms—that is, for the existence of discrete, gene-like hereditary units in bacteria—then these organisms might prove to be invaluable material not only for the study of physiological genetics, but also for an attack on the problems of gene structure and mutability (4, 5).

The case would necessarily rest on analogies, some of which may be listed as follows: (1) permanence of the mutated character; (2) "spontaneous" occurrence, independent of specific environmental stimuli; (3) definite rate of occurrence; (4) independence of different mutations in the same organism; (5) inducibility by agents known to produce mutations in higher organisms; (6) reversibility, showing that the mutation is not necessarily a loss, caused by unequal division or some similar process; (7) physiological effects, furnishing proof that bacterial mutations can bring about the same type of metabolic changes known to be produced by gene mutations in higher organisms.

One difficulty of bacterial genetics is that of recognizing a given mutational step, since the same character may be affected by a number of different mutations, which we cannot attribute to different genetic loci by classical genetic tests. Hence the necessity of identifying each mutation by as many of its phenotypic effects as possible.

Another peculiarity of bacterial genetics is that we generally do not deal with the properties of single individuals, but only with those of large "clones" (colonies, single-cell cultures), each derived from one individual whose characters must

be retraced from those of the clone. We must therefore take into account intracolony variability. Cases of high mutation frequency are generally unsuitable for the study of the mutation process, since they are likely to be balanced by reverse mutations; our visible clones will then contain mutant and reverted types in genetic equilibrium. These considerations have been discussed in greater detail by Delbrück (8).

More convenient are cases with low mutation frequency, since these need not necessarily be balanced by either reversion or adverse selection. In these cases the mutants can be detected, however, only if we can select them out in a suitable environment. Mutants capable of growth in media "incomplete" for the wild type (22, 19) are in this category. Other such mutations are those producing increased resistance to some destructive agent.

We shall discuss in this paper the latter group of mutations, on the basis of work on bacterial resistance to bacteriophages, antibiotics, drugs, and radiation (18, 11, 1, 2, 9, 24, 21).

ORIGIN OF RESISTANT VARIANTS

The resistant variants are generally isolated as colonies which appear after the parent strain has been plated in the presence of a destructive agent. The question then arises: were the cells that gave these colonies resistant before the plating, or have they acquired hereditary resistance as a response to the action of the antibacterial agent? Unappealing as the second alternative may be to classical genetic thought, the question is by no means idle. Considerable evidence has been interpreted as supporting the idea that environmental conditions, in particular nutritional or inhibitory stimuli, may alter the metabolic machinery of the bacterial cell, not only temporarily, but in a permanent hereditary way (14, 13). It may be conceivable that a small fraction of the cells exposed to an antibacterial agent respond to its action with a specific change in properties, and that this change is somehow transmitted to the offspring.

Mutational origin of the resistant variants, independent of the action of the destructive agent, can only be proved either by direct isolation of resistant substrains before the treatment (7), or by establishing the presence within sensitive cultures of "clones" of resistant bacteria, each clone stemming from a mutation that occurred during the growth of the cultures prior to the test for resistance (18). If we define mutation rate as the probability that a

bacterium will mutate in a given interval of time, the frequency of mutations will be proportional to the number of bacteria present. Each mutation gives origin to a "clone" of mutants; the earlier the mutation occurs, the larger the clone derived from it will be at the moment of the test. If the mutants grow at the same rate as the normal bacteria, clones of a certain "size" will be twice as frequent as those of double that size. The expectation should be, therefore, that, of the mutants present in a culture at any one time, equal numbers stem from mutations that occurred in each of the previous generations. This situation, however, is never realized in actual cases. In any actual culture, the likelihood

same culture. A detailed analysis of these considerations has been given by Luria and Delbrück (18).

These expectations can best be tested in the case of resistance to bacteriophages. The phage-resistant variants are easily detected and counted in a mixture with sensitive cells; they neither grow the phage nor adsorb it, and their resistance is a permanent new character of the strain.

As an example, Table 1 (from 18) shows the results of the enumeration of bacteria resistant to phage T1 in platings from similar cultures of *Escherichia coli*, strain B. The presence and type of fluctuations in the frequency of mutants provide strong confirmation of the mutational origin of re-

TABLE 1. DISTRIBUTION OF THE NUMBERS OF BACTERIA RESISTANT TO PHAGE T1 IN SERIES OF SIMILAR CULTURES OF SENSITIVE BACTERIA

Experiment No.	22		23	
Number of cultures	100		87	
Volume of cultures, cc.	.2*		.2*	
Volume of samples, cc.	.05		.2	
	Resistant Bacteria	Number of Cultures	Resistant Bacteria	Number of Cultures
	0	57	0	29
	1	20	1	17
	2	5	2	4
	3	2	3	3
	4	3	4	3
	5	1	5	2
	6-10	7	6-10	5
	11-20	2	11-20	6
	21-50	2	21-50	7
	51-100	0	51-100	5
	101-200	0	101-200	2
	201-500	0	201-500	4
	501-1000	1	501-1000	0
Average per sample	10.12		28.6	
Variance (corrected for sampling)	6270		6431	
Average per culture	40.48		28.6	
Bacteria per culture	2.8×10^8		2.4×10^8	

* Cultures in synthetic medium.

of occurrence of early mutations is small, because not enough individuals are present. When the population reaches a size where mutations begin to occur, the frequency of their occurrence will be subject to tremendous fluctuations owing to their small average number. In a large number of trials (series of similar cultures), therefore, we must expect to find corresponding large fluctuations of the frequency of mutants; the presence of these fluctuations is a direct consequence of the clonal distribution of the mutants. When the proportion of variants in a series of similar cultures shows such large fluctuations, we may interpret them as proof of a clonal distribution, hence of a mutational origin of the variants.

Such fluctuations among similar cultures would not be expected if resistance were actively produced by the antibacterial agent. The fluctuations should not be greater than those among samples from the

sistance. A refinement of this type of analysis would be afforded by a comparison of the actual distribution of the number of variant individuals with a distribution calculated from the assumptions of the mutation hypothesis. Mathematical difficulties have been encountered, however, in attempts to calculate the theoretical distribution.

It is important to notice in Table 1 (Exp. 23, in which the whole contents of the cultures were tested) the presence of large numbers of cultures containing one mutant only. This suggests that the spontaneous mutations may be phenotypically expressed without a lag longer than one cell generation.

The "fluctuation" test has been applied to a variety of cases—resistance of *E. coli* B to several phages (11) and to radiation (24); resistance of *Staphylococcus* 313 to penicillin (9), and to sulfathiazole (21)—with results of the same type, indicat-

ing that in all these cases resistance arises as the result of mutations occurring before the test. The data of Lewis (16) on *Escherichia coli mutabile* show that similar fluctuations between cultures were present in the numbers of lactose-fermenting cells. Large fluctuations in the time of appearance of fermentation variants were also present in experiments of Kristensen (15). The mutational origin of bacterial variants is well supported by all these observations.

The type of test described above shows only that the variants were already a genetically modified type before the test. It is possible that in some cases the specific environment, besides selecting out the mutants, may also act by rendering phenotypical a new character otherwise masked by some cytoplasmic effect. In a sense, this may be considered to be true of mutations affecting the production of adaptive enzymes. In the case of mutations to resistance to antibacterial agents, similar instances might be difficult to detect.

MUTATION RATES

In work on bacteriophage resistance, Luria and Delbrück (18) have chosen as a definition of the mutation rate the probability of mutation per bacterium per physiological time unit (generation). This definition is satisfactory as long as it can be proved that mutations occur only during growth of the bacteria, and that their frequency is proportional to the physiological rather than to the astronomical time. These conditions appeared to be fulfilled in our experiments on phage resistance (mutation rate independent of generation time in different media, no increase in mutants after maximum growth).

Several methods have been proposed for estimating the mutation rate. Since mutations occur independently of each other, when there are only a few they will be distributed among different cultures in a Poisson distribution, with the probability of any given number of mutations occurring in one culture depending only on the average number of mutations per culture.

The average number of mutations per culture, and hence the mutation rate, can be calculated from the number of cultures having no mutants and from the number of bacteria per culture. If in a series of C similar cultures, each containing N bacteria, a fraction C_0/C proves to contain no mutant (the whole contents of the cultures must obviously be tested), the mutation rate, a , can be obtained:

$$(1) a = - \frac{\ln 2 \times \ln (C_0/C)}{N}$$

This method, although it requires careful planning of the experiments, has the advantage of not introducing the actual numbers of mutants and thus avoiding complications due to possible selection.

Another method that allows direct measurement

of mutation rates without making use of the numbers of mutants consists in applying the antibacterial agent (phage) to bacteria growing on solid medium without disturbing the formation of microcolonies (10). One colony appears for each mutation that has occurred. The difficulty here lies in precise estimation of the number of bacteria present at the moment of the test.

Mutation rate can also be calculated from the actual number of mutants, estimating from this the number of clones present. One must take into account that in cultures of limited size mutations are likely to occur only during the last part of their growth. If, for example, the mutation rate is 10^{-7} , mutations are not likely to occur, in actual cultures, until the population reaches a size of the order of 10^7 cells. Introducing the necessary corrections, the mutation rate can be estimated from the average number, r , of mutants from C similar cultures:

$$(2) r = \frac{aN}{\ln 2} \ln \frac{aN}{aNC}$$

A diagram for the use of this formula has been given by Luria and Delbrück (18). Mutation rates calculated by formula (2) are likely to be inaccurate because of the fluctuations in frequency of occurrence of the early mutations; in particular, they will be too high whenever one or more of the unlikely early mutations happens to occur. Important corrections should be introduced if the growth rates of normal and mutant types are different. Altogether, our present methods of estimating mutation rates are far from satisfactory.

Some values of mutation rates calculated with formulas (1) and (2) are given as examples in Table 2. These values are subject to the further limitation that all mutations giving the same phenotype for the character investigated are lumped together, and that in some cases it is likely that not all mutants present in a sample are detected. The orders of magnitude, however, are probably correct.

Even for the same wild-type strains, mutation rates for different mutations to resistance vary over a wide range. That they are generally very small is to be expected; in searching for stable resistance to destructive agents, we particularly select for rare mutations, since if stable mutants occurred very frequently they would generally have displaced the parent type, unless checked by strong adverse selection. Much higher mutation rates may be found for mutants that also show high rates of reversion.

INDEPENDENT MUTATIONS

It has already been mentioned that the same bacterium can undergo different mutations. This is clearly seen in the case of resistance to bacteriophages. Working with *Escherichia coli* B and bacteriophages T1-T7, one finds a series of bacterial mutations, each producing resistance to one, two,

or more phages. Indicating a mutant from B that is resistant to phage *n* by the symbol B/*n*, we have for instance the mutants: B/1; B/1, 5; B/6; B/3, 4, 7. From each of these mutants one can obtain other mutants with additional resistance to other phages: B/1/6; B/6/3, 4, 7, etc.

The successive mutations are clearly independent. A strain that has undergone a certain mutation will generally give the same mutations to resistance to other phages as the parent wild-type strain. Within the limits of precision of the available methods of measurement, the rates for the same mutation in the parent strain and in any of its mutants appear

could decide by what mechanism resistance to unrelated phages is produced in one mutational step. The answer to this question might be of the greatest importance, also, in relation to the mechanism of bacteriophage action.

A possible interpretation has been suggested by E. H. Anderson's work on the nutritional requirements of some phage-resistant mutants (1, 2). Anderson found that a number of B/1 mutant strains from *Escherichia coli* B differed from the wild type also in being unable to grow in a minimal synthetic medium. The metabolic disturbance was identified as inability to synthesize *l*-trypto-

TABLE 2. MUTATION RATES

Organism	Mutation	Reference	Mutation Rate per Bacterium per Generation	
			Formula (1)	Formula (2)
<i>Escherichia coli</i> B	Resistance to phage T1	(18)	3×10^{-9}	1.7×10^{-8}
	Resistance to phage T1	(11)	7×10^{-9}	2.7×10^{-8}
	Resistance to phage T3	(11)	5×10^{-8}	1.2×10^{-7}
	Resistance to phage T6	(11)	5×10^{-8}	$\sim 2 \times 10^{-7}$
	Resistance to radiation	(24)		$\sim 10^{-6}$
<i>Staphylococcus aureus</i> 313	Resistance to penicillin, one-step	(9)		$\sim 10^{-7}$
	Resistance to sulfathiazole, one-step	(21)	$\sim 10^{-9}$	

to be equal. For example, the mutations B → B/1, and B/6 → B/6/1 occur with the same frequency (11).

The same holds true for other types of mutation as well. The mutant B/*r* resistant to radiation (24) has the same pattern of mutability to phage resistance as the wild-type B.

These results provide evidence of the independence of different mutations in bacteria, and agree with the hypothesis of the existence of discrete, mutable, gene-like units.

MULTIPLE EFFECTS OF MUTATIONS

In cases of resistance to drugs and antibiotics, the resistance is often very specific; cross-resistance is limited to related substances. In the case of phage resistance, however, multiple resistance to several phages may appear as one mutational step. The phages to which resistance can be produced by one mutation are often unrelated as judged by their size, structure, serological specificity, and growth characteristics (8). It is easy to prove, on the basis of the mutation rates, that the multiple resistance does not result from chance occurrence of two or more independent mutations in the same bacterial line. Moreover, resistance to the same group of phages can be reached in one or two mutational steps. It is clear, therefore, that tests of resistance to a limited number of phages are not sufficient to characterize the genotypes of a group of bacterial mutants.

It would be an important step forward if we

phane, which therefore became a required nutrilit for these strains. Moreover, these strains exhibited another disturbance in their nitrogen metabolism, reflected by their inability to utilize inorganic nitrogen in the absence of at least one of a series of amino acids.

Additional inability to synthesize the amino acid proline was found by Anderson in a strain B/1/3, 4, 7. Other phage-resistant strains that fail to grow in a minimal medium have also been encountered.

Anderson (1) interpreted his findings as indicating the existence of common steps in the chains of reactions leading to the synthesis of an essential metabolite and of substances needed for phage sensitivity, the mutation blocking one of the enzymatic reactions. As an extension of this interpretation, it was suggested that multiple resistance to various phages acquired in one mutational step results from the blocking of a reaction common to the syntheses of substances needed for growth or adsorption of the phages. Common steps could occur in the chains of reactions leading to sensitivity to unrelated phages. Different mutations producing resistance to more phages would probably produce blocks at different levels of the series of reactions.

Since bacteria resistant to a certain phage are often sensitive to some mutants from that phage, the "blocks" in the chains of synthetic reactions are likely to alter, rather than suppress completely, the synthesis of substances necessary for phage sensitivity.

It was thought (17) that the mutational changes might simply involve slight alterations in the configuration of the bacterial receptors for phage adsorption, which were supposed to react with the phage by simple coming together of complementary surface structures. Mutational alteration of the surface receptors could be brought about either by a primary change in the receptor itself or by an alteration in the structure of a gene-like center acting as template for the receptor, without involvement of any long reaction chain. The association of specific metabolic deficiencies with phage resistance seems to contradict this idea. Moreover, the finding of T. F. Anderson (3) that certain amino

phage-resistant mutant of the usual type. Unfortunately, the relation between the two types of resistance was not thoroughly analyzed. The indications were, however, that phage resistance in the acridine-resistant strain was caused by a change in an acridine-sensitive mechanism involved in phage growth. Interestingly enough, both antiphage action of the acridines and phage resistance of the acridine-resistant strain could be antagonized by the addition of ribose nucleic acid.

The idea that the usual type of phage resistance is due to a block in a chain of synthetic reactions would be convincingly proved if it were possible to restore sensitivity to a resistant mutant by supply-

TABLE 3. ONE-STEP MUTANTS

Phenotype	Phages									Tryptophane Requirement	Generation Time, Minutes, in Broth at 37° C.
	T1	T5	T2	T6	T4	T3	T7	T7h	T7h'		
Wild type	S	S	S	S	S	S	S	S	S	-	19-20
Frequent mutants											
a	R	S	S	S	S	S	S	S	S	+	19-20
b	R	R	S	S	S	S	S	S	S	-	19-20
c	S	S	S	R	S	S	S	S	S	-	19-20
d	S	S	S	S	R	R	S	S	S	-	25-26
e	S	S	S	S	R	R	R	S	S	-	25-26
f	S	S	(S)	(S)	R	R	R	R	S	-	29-30
Complex mutants											
g	R	S	(S)	(S)	R	R	R	R	S	+	29-30
h	R	R	(S)	(S)	R	R	R	R	S	-	28-29
i	S	S	R	R	R	R	R	S	S	-	28-29
l	R	S	R	R	R	R	R	S	R	+	40

* The braces indicate serological relationship between phages.

acids may act as necessary cofactors for phage adsorption indicates that the latter process is likely to involve some rather complex enzymatic activity of the phage particle on the bacterial surface.

We found recently that some of the B/3, 4, 7 mutants, besides not adsorbing phages T3, T4, and T7, also show a partial inability to grow phages T2 and T6. They adsorb these phages like normal wild-type bacteria, but 80-90% of the infected bacteria fail to liberate any phage at all. Similar observations were also made by Hershey (personal communication). This phenomenon, which needs further investigation, indicates a relation between adsorption capacity and ability to grow phages, and supports the idea that both phenomena involve chains of reactions variously linked.

Additional evidence for relating phage resistance to specific changes in enzymatic reactions is offered by recent results of Fitzgerald and his collaborators (12). It was found that some acridines exerted a specific inhibition on phage growth in the bacterial cell, and that a variant resistant to one of the acridines was almost as resistant to phage as a

ing its medium with the intermediate metabolite whose synthesis is supposedly blocked, and whose absence is assumed to be responsible for the phage resistance. Such cases have not yet been found.

Some additional information on multiple resistance has been derived from a category of rare mutants with unusual combinations of resistance to various phages (11). We have recently isolated a number of these "complex" mutants, and attempted a further characterization of their phenotypes, comparing them with those of the more common mutants. This was done by taking into account sensitivity to phages T1-T7 and to some of their host-range mutants (active on some of the resistant bacterial mutants), nutritional requirements, and growth rates, which will be considered later. The complex mutants were isolated by plating large numbers of sensitive bacteria in the presence of suitable mixtures of phages. Most of them occur with mutation rates of the order of 10^{-10} . The results of some of these tests are summarized in Table 3.

We see that certain basic groupings of characters

is corroborating evidence of its spontaneous, mutational origin. The successive mutations to resistance might represent either similar changes in a series of gene-like units, or successive changes in the same unit, or independent genetic changes each affecting one of the metabolic processes on which the antibacterial agent may act.

A study of sulfonamide resistance in *Staphylococcus* (21) indicates that the last alternative is probably correct. A number of different mutations are involved in acquisition of this resistance, most of the recognized ones occurring at comparable rates (between 10^{-9} and 10^{-10}). These mutations can be differentiated first of all by the degree of resistance produced, expressed by the drug concentration withstood by the mutant. Even more important is the fact that different mutants with increased sulfonamide resistance differ in other correlated characters, particularly in the production of an extracellular sulfonamide antagonist apparently identical with *p*-aminobenzoic acid. Some of the resistant mutants release during growth up to 30 times more p.a.b. than the parent strain, while others do not produce any increased amount of it. The same mutation affecting p.a.b. production may occur at the same rate either in the parent strain or in mutants with already increased resistance but without increased p.a.b. production.

The simplest explanation is that sulfonamides act as inhibitors of a number of metabolic reactions (whether all involving p.a.b. or not is immaterial for our problem). Successive mutations to resistance produce changes in different enzymatic mechanisms, the residual level of sensitivity being determined by the most sensitive mechanism left.

In the case of penicillin resistance (9), the occurrence of mutations producing different degrees of resistance indicates a similar situation. That penicillin affects a number of different enzyme systems of the bacterial cell is indirectly indicated by data of Treffers (23) on growth inhibition brought about by associations of penicillin with each of a number of metabolic poisons in noninhibitory amounts.

The acquisition of resistance to drugs and antibiotics by mutation in bacteria seems, therefore, to fall in line with the idea of genetic determination of single enzyme reactions by individual genes. It is interesting to note that, in the case of resistance to radiation in *E. coli* B, Witkin (24) found only one mutational step increasing resistance. All attempts to select mutants having higher resistance failed. It is likely that only one of the synthetic mechanisms responsible for sensitivity to radiation can be modified by a nonlethal genetic change.

The increased production of p.a.b. by sulfonamide-resistant mutants of *Staphylococcus* exemplifies a metabolic change increasing the extracellular production of a metabolite. P.a.b. is not a required metabolite, and is normally produced by the organ-

ism. It is possible, on the one hand, that p.a.b. accumulates because of a block in a sulfonamide-sensitive reaction that utilizes p.a.b. Provided an alternative metabolic pathway for growth were available, such a block would be expected to produce sulfonamide resistance. It is possible, on the other hand, that an actual increase in p.a.b. synthesis is caused by the mutation. That positive biochemical mutations may be responsible for resistance to antibacterial drugs seems indicated by the increased formation of pantothenate by *C. diphtheriae* in the course of its becoming pantoyltaurine-resistant and independent of pantothenate for growth (20).

GROWTH OF THE RESISTANT MUTANTS

The resistant mutants often show definite differences in growth characteristics from the sensitive wild-type parents, which can be used as additional criteria for identification of a given mutational step.

In the case of phage-resistant mutants, some mutations are correlated with definite changes in growth rate (generation time). Some of the data for the growth of one-step mutants in nutrient broth at 37° C. are included in Table 3. The growth rates of the mutants range from the same value as for wild-type to values more than twice as great. These differences again indicate that the mutations produce profound changes in the metabolic processes of the bacterial cell.

It was found that when successive mutations are accumulated in the same bacterium the growth rate is always determined by the mutation that by itself gives the slowest growth.

We can see in Table 3 that those complex mutants that appear to be the superposition of two simple mutant types also have the same generation time as the slower simple mutant type included. This agrees with the suggestion that they actually originate by combined mutations. Others of the most complex mutants have a much slower division time, probably indicating a more profound alteration of their synthetic abilities.

The effects on growth of mutations to resistance are not limited to changes of generation time in the logarithmic phase. All phases of growth may be affected. Among mutants resistant to phages or to penicillin, we found some having longer or shorter lag phases, others with higher or lower maximum titers than the wild-type strain.

Of a large number of mutants whose growth was measured under comparable conditions, a majority grew less well than their wild-type parents. Only a few seemed to grow as well or better. This seems to agree with the general principle that most mutations arising in well-established wild-types produce less favorable phenotypes.

Complications may arise, however, when one attempts to predict the growth of parent and mutant strains in mixtures on the basis of their growth in isolated cultures. Production of diffusible growth

inhibitors or stimulants, or differential rates of utilization of some nutritive, may greatly alter the picture. For instance, we found that the viable counts of a penicillin-resistant mutant in mixed culture with the parent strain began to fall long before the end of the growth phase for separate cultures of either strain. Other instances of interactions of this type have also been encountered. In different phases of the life of a bacterial culture either the parent type or its mutants may be favored, and only detailed study of individual cases can clarify the most complex situations. The indications are, however, that many cases of bacterial variation, including some of the most complex types of so-called life cycles, can be explained in terms of the simplest hypothesis—mutation followed by selection within the resulting mixtures of phenotypes (6).

SUMMARY

Bacterial mutations to resistance have provided favorable material for the quantitative study of bacterial mutability. The mutations discussed in this paper present suggestive analogies with gene mutations in higher organisms, although the similarity of the genetic systems involved can only be considered, for the time being, as a useful working hypothesis. The problems of determination of mutation rates, of identification of single mutation steps in strains with different genotypes, and of multiple effects of mutations have been discussed.

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DISCUSSION

KIDD: I wonder if Dr. Luria has seen a resemblance between the complex races of phage-resistant bacteria with which he has been working and the strains of drug-fast trypanosomes which have interested parasitologists since the time of Ehrlich. While the genesis of the drug-fast strains of trypanosomes has not perhaps been particularly well studied as judged by the criteria of critical quantitative genetics, the fact has long been known that they represent more or less stable heritable variations in microorganisms. Furthermore, the work of Warrington Yorke and his collaborators and that of Jancsó and others has made it abundantly plain that the lethal effects of drugs on trypanosomes depend upon their fixation by the parasites: arsenical and acridine compounds, for example, are absorbed by strains of normal trypanosomes, which then succumb to their

effects; but the compounds are not absorbed by the drug-fast strains, which in this way resist their action. From a study of the phenomena of drug resistance, moreover, Frank Hawking has distinguished four different kinds of receptors in trypanosomes—those for arsenicals and acriflavine, for parafuchsin, for diamidine compounds, and for Bayer 205—and he has studied the relationships among them.

It seems to me that the parasitologists have managed to acquire a noteworthy comprehension of the phenomena with which they were confronted—the practical implications for chemotherapy included—and it may become manifest that the principles involved are much the same as those with which Dr. Luria is dealing.

SONNEBORN: The finding of a single mutant colony when an entire culture is plated out need not necessarily indicate that the mutation was brought to phenotypic expression at once in the absence of further cell reproduction. Suppose the mutation had occurred several cell generations earlier, with a lag of some cell generations between mutation and phenotypic expression and with variation among the progeny of the mutated cell in the number of cell generations preceding phenotypic expression. In *Paramecium*, for example, precisely such variation is observed. Under such conditions a single descendant cell might resist phage and the others be destroyed. It would then be difficult to distinguish this from cases of immediate phenotypic expression.