gm.) per square meter. The nonassimilated material (feces) does not change trophic levels but is available to other primary consumers such as bacteria or detritus feeders. The annual energy flow of the snail population was estimated to be about 290 Calories/M^2/year, but during the summer the average standing crop of 700 snails/M^2 assimilated only about twice as much as the average population of 10–20 grasshoppers/M^2 (Fig. 3). While it would appear that both populations utilize only a small percentage of the total net primary production of the ecosystem it should not be assumed that food is nonlimiting. The maximum portion of the net production which is actually available to the populations has not yet been determined, nor has the utilization of competing primary consumers been considered. The fact that growth rate per individual in snails declined with increasing density in the field suggests that the rate at which nourishment can be ingested and assimilated on a square meter of marsh is not unlimited.

It is evident that populations which differ greatly in life history characteristics, age structure, and metabolic rate cannot be compared on the basis of numbers and biomass (i.e., "standing crop") alone. However, through the common denominator of energy flow valid comparisons can be made, and the true role of the populations in the community can be evaluated. Energy flow analysis is the logical first step in finding out how populations really function in nature.

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[1] Odum, Howard T., *Ecol. Monogr.*, **27**, 55 (1957).

[2] Teal, John M., *Ecol. Monogr.*, **27**, 283 (1957).

[3] Smalley, Alfred E., Ph.D. Thesis, University of Georgia, 1959.

[4] Ivlev, V. S., *Biochem. Ztschr.*, **275**, 49 (1934).

[5] Burkholder, P. R., and G. H. Bornside, *Bull. Torry Bot. Club*, **84**, 366 (1957).

[6] Pomeroy, L. R., *Limnol. and Oceanogr.*, **4** (in press).

THE DIFFERENCE BETWEEN SPONTANEOUS AND BASE-ANALOGUE INDUCED MUTATIONS OF PHAGE T4

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This paper describes studies about the induction of reverse mutations by two base analogues, 2-aminopurine (AP)[1] and 5-bromo-deoxyuridine (BD). It will be shown that mutants of phage T4 induced by these base analogues in the forward direction can also be induced to revert. In contrast, proflavine induced and most spontaneous mutants cannot be induced to revert by these base analogues, although

they revert spontaneously. Superimposed on this fundamental difference between base analogue inducible and noninducible mutations is a further difference in the relative effect of AP and BD. A further examination of spontaneous and induced revertants indicates that most arise by a back mutation to the standard genotype. A molecular explanation of these results is possible, and provides a better understanding of spontaneous mutations.

MATERIALS

Phages—All mutants examined for the induction of revertants have the *r*II phenotype; i.e., they lack the function necessary for phage growth in bacteria K, and they form *r*-plaques on bacteria B. Their mutations are located in the *r*II region of the phage T4 genome; their isolation and genetic properties have been decribed previously.[2–4]

Bacteria, broth, and *synthetic* medium, see reference 3.

H-Medium, synthetic medium, plus 20 μg/ml histidine, plus 0.25 μg/ml thiamine.

F10 Medium, per liter H-medium 20 mg L-glycine, 20 mg L-methionine, 10 mg L-leucine, 10 mg L-valine, 10 mg L-serine, 10 mg adenine sulfate, 10 mg guanine sulfate, 10 mg uracil, 0.2 mg Ca pantothenate, 0.2 mg pyridoxine, 10 μg Vitamine B12.

Aminopterin (gift of R. B. Angier, American Cyanamid Company).

2-Aminopurine and *5-bromodeoxyuridine*, Sigma Chemical Company. For spot tests, solutions in broth of 10 mg/ml and 5 mg/ml, respectively, were used.

RESULTS

Reversion Rates in Liquid Medium.—Revertants of *r*II-type mutants can be detected, selectively, when the phages of an *r*II-type stock are plated on bacteria K. In most cases plaques are of the wild phenotype; exceptions will be discussed later. For a quantitative measurement of reversion rates, it is best to prepare lysates from a small inoculum of given number (e.g., 100 phages). The introduction of a revertant, already present in the previous stock, is then rare, and can easily be detected by an exceptionally large proportion of revertants in the lysate, when compared to parallel cultures. Fluctuations in the proportion of revertants also arise because the corresponding mutations may occur early or late in phage growth, and the revertants can multiply after their formation. An unusually great fluctuation of this kind will be called a "jackpot." The average ratio of revertants to viable phages (assayed on B), for stocks grown from an inoculum of 100 phages, is called the *reversion index*.

The *induction of revertants* is measured by comparing the reversion index of phage stocks grown in the presence and absence of the mutagen.

The following procedure was used for the measurement of reversion indices with and without induction by AP and BD: 400 phages (in synthetic medium) were added to 20 ml. of the growth medium, containing 10^8/ml bacteria. The content was immediately equidistributed, to 4 test tubes. The tubes were incubated at 37°C for 24 hr, with occasional shaking; chloroform was then added. The lysates were plated on B and K to determine the phage titer and the frequency of revertants.

For the *induction by AP*, bacteria B-97 grown in H medium + 30 μg/ml adenine were added to H medium + 15μg/ml adenine + 500 μg/ml AP.

For the corresponding *control*, the same medium without AP was used.

For the *induction by BD*, bacteria B grown in F 10 + 20 μg/ml thymine were added to F 10 + μg/ml thymine + 50 μg/ml aminopterin + 50 μg/ml BD.

For the corresponding control the same medium was used, containing 50 μg/ml thymidine in the place of BD. The thymidine addition was found necessary since the phage titer otherwise decreased instead of increasing, although the bacteria continued to grow. This shows both the strong inhibition of thymine production by aminopterin and the ability of BD to serve as growth factor in the place of thymidine.

TABLE 1

REVERSION INDEX AFTER PHAGE GROWTH WITH AND WITHOUT BASE ANALOGUES

	Mutant	Reversion Index: With AP	Reversion Index: Without AP	Ratio, with/without AP	Reversion Index: With BD	Reversion Index: Without BD	Ratio, with/without BD
Spontaneous	r111	1,511	265	5.7	408	301	1.4
	r114	0.15	0.11	1.4	0.49	0.29	1.7
	r117	6.6	2.0	3.3	3.0	1.3	2.2
	r131	5.2	0.93	5.6	1.4	1.5	0.9
	r157	1,490	592	2.5	890	933	1.0
	r215	1.04	0.002	500	0.48	0.002	240
BU induced	N11	408	0.53	770	10	0.18	56
	N12	1,705	0.02	95,000	2.3	0.01	23
	N21	98	0.014	7,000	0.14	0.03	4.7
	N24	796	0.63	1,260	2.4	0.29	8.3
	N29	1,705	0.054	32,000	0.5	0.05	10
	N34	270w 48ti	0.09w 20 ti	3,000w 2ti	0.33w 65 ti	0.04w 21 ti	8w 3ti
	N101	559	≤0.01	560,000	2.58	≤0.001	2,600
AP induced	AP12	31	0.14 +2 ti	220	88	0.13 +2 ti	680
	AP61	86	0.16	540	4.7	0.05	940
	AP72	74	0.2	370	3,070	0.15	20,000
	AP133	198	5.5	36	5,130	15	340
	AP156	4.2	0.006	700	23.1	0.002	11,500

The table gives the *reversion index*, averaged (arithmetically) over parallel stocks, in *units* 10^{-6}. The fluctuations from stock to stock are usually in the range of a factor of 5. Excessive fluctuations (jackpots) have not been used for determining the average. The ratio of the reversion index with and without the mutagen is given in the columns "*ratio*." The spontaneous reversion index of mutant N101 is so low that its absolute number could not be determined and only a limiting value can be given, according to the number of phages plated. For the determination of the ratio with/without base analogue the K/B value 0.001 has been used in this case for both AP and BD. The mutants AP12, AP133, and N34 are exceptional in giving rise to two kinds of spontaneous revertants, which on K produce wild (w) and tiny (ti) plaques, respectively.

A typical example for the *results* obtained is given in Table 1. The table includes one mutant for each site of high mutability ("hot spot")[2] and some others of special interest.

The reversion indices of the *controls* obtained in the AP-less and the BD-less media agree quite well, considering the fluctuations in individual stocks.

The increase of the average reversion index, due to the presence of AP or BD in the medium, varied with the mutant and the base analogue used. AP had the largest effect, and increased the reversion index of N12 and N101 by a factor of about 10^5. For some mutants the effect of AP was larger than that of BD; in other cases the reverse was observed.

Five of the spontaneous mutants, belonging to five spontaneous hot spots,[2] showed a small increase of reversion index with AP, and nearly no increase with BD. This effect, however, is so much smaller than the mutagenic effect observed for other mutants that it appears to reflect only the selective action of AP and BD upon phage multiplication. AP has indeed been found[3] to suppress lysis inhibition; it may therefore suppress the slight selection against revertants, which is caused by

lysis inhibition during the phage growth in several bacterial cycles. The absence of reversion induction for these spontaneous mutants is furthermore shown by the negative result of spot tests (see later).

The spontaneous mutant *r* 215 has been included in this list because it constitutes an exception among the spontaneous mutants, as will be seen later. Its spontaneous reversion index is exceptionally small (as compared to other spontaneous mutants), and both AP and BD increase the index by a factor of about 500.

Of special interest is mutant *N*34 which spontaneously gives rise to some revertants of standard phenotype (w-plaques) and many "partial revertants" of tiny phenotype (ti-plaques). During growth in AP (and less efficiently in BD) the frequency of standard type revertants increases 1000-fold while the frequency of partial revertants does not change significantly.

Spot Test for the Induction of Reversions.—The measurement of reversion rates in liquid medium is accurate but cumbersome, since several parallel cultures must be grown. A qualitative decision, however, for the presence or absence of reversion induction can be obtained for each mutant by means of a simple spot test.[5,3]

In this test different numbers of phages (around 2×10^7) are plated on cold T plates together with a mixture of about 2×10^8 bacteria K and 2×10^7 B. The addition of B enables some of the *r*II-type phages to multiply and undergo mutations. When all bacteria B are lysed, only the revertants continue to multiply in K and give rise to plaques. The conditions applied seem to be optimal for the detection of small mutagenic effects by AP and BD; high mutagenic effects can also be detected when less bacteria B are added. A small drop containing 10 mg/ml AP was placed on one side of the plate and another drop containing 5 mg/ml BD on the opposite side. The plates were immediately placed in the cold for 2 hr in order to permit a slight diffusion of the base analogues. This cold storage is not necessary for high but useful for small inductions by BD, since the diffusion produces a gradient of BD concentration. Phage growth is inhibited in the center of the BD spot but at the rim mutations are expressed by many plaques. After subsequent incubation in 37°C the plates were inspected. The induction of mutations showed up by a large increase of plaques in the region of the spotted base analogue. In general, AP was more efficiently mutagenic than BD.

Besides being simple the spot test has additional advantages. Instead of comparing the reversion rates of different growth tubes one observes on the same plate the induction of reversions above the spontaneous rate and directly compares the relative effect of AP and BD. The problem of jackpots is eliminated, since each revertant which arises early enough in phage growth gives rise to one and only one plaque. Moreover the number of plaques cannot increase when the base analogue merely affects the degree of lysis inhibition or otherwise preferentially inhibits the development of certain phages; each plated phage revertant can produce only one plaque.

The spot test is quite sensitive, as shown by a comparison to the quantitative measurements in liquid medium. It rarely fails and then only for weak induction (1) when the induced reversion index is very small, (2) when the mutant is rather leaky, (3) when in addition to rare inducible reversions another (tiny) type of frequent noninducible reversions is produced (as for the mutants of site N 34). This rare failure (mostly for BD induction) does not affect our analysis since for all mutants found base analogue inducible, at least one of the two base analogues causes a strong, easily detectable mutagenic effect. Only very rarely can base analogue induction have escaped detection.

Using this spot test the induction of reversions was determined for all available mutants of the *r*II-type. For each mutant the test was repeated several times, varying also the number of phages plated. The result for spontaneous, AP, and BD induced mutants is given in Figure 1, and summarized in Table 2.

Most striking is the difference between spontaneous and base analogue induced

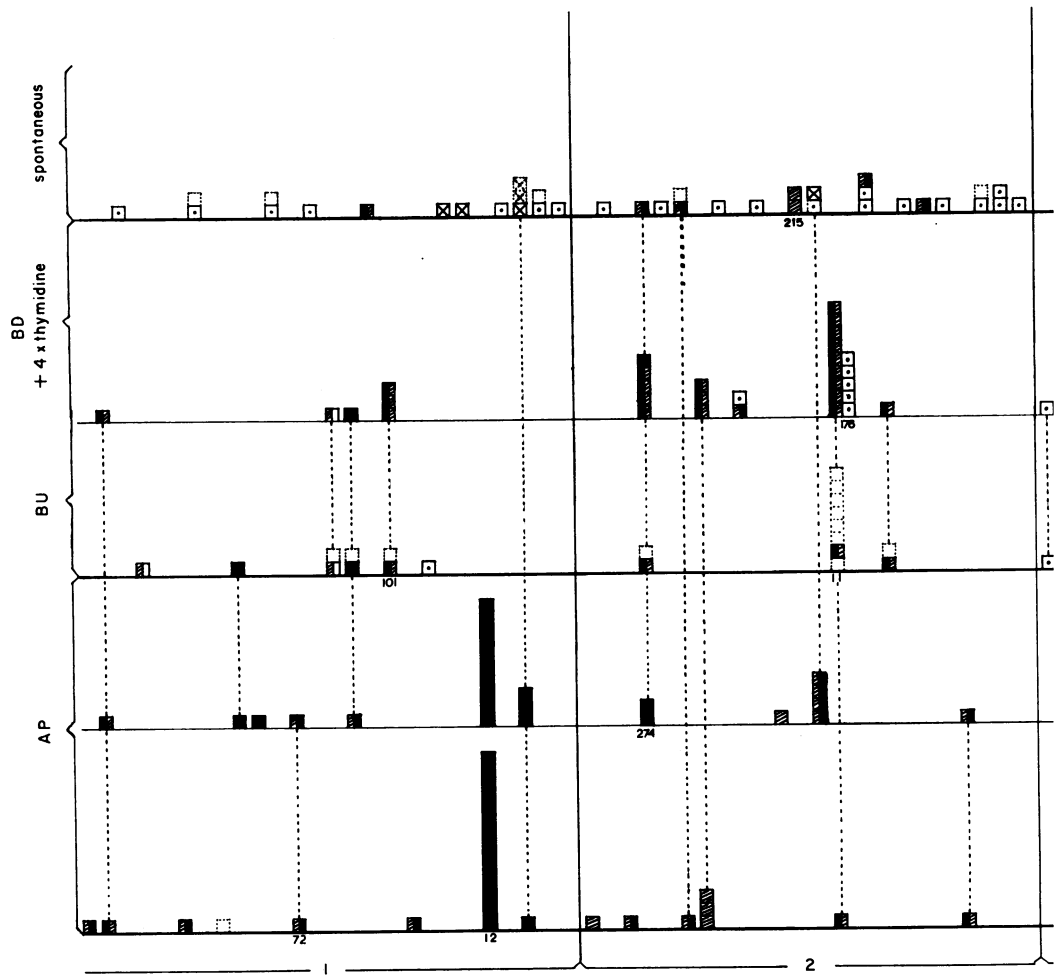


FIG. 1.—Genetic map of the *r*II region of phage T4 showing the approximate location of mutations for independently isolated *r*II-type mutants. Mutations induced in 5 different experiments are sketched on 5 different horizontal lines.

First horizontal line: spontaneous mutations (*r*) (see Benzer and Freese[2]).

Second horizontal line: mutations (*N*) arising under the action of BD plus 4 × as much thymidine (see Freese[3]).

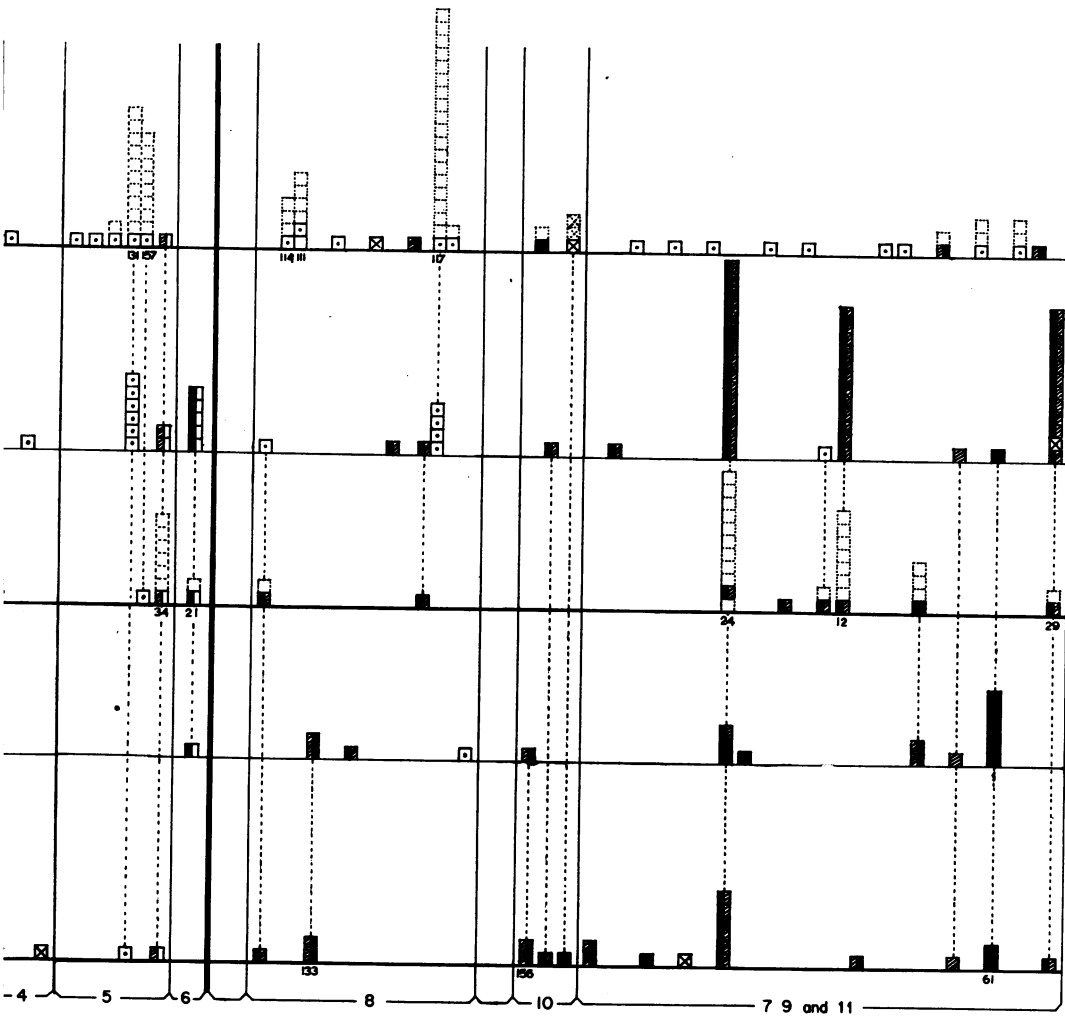
Third horizontal line: mutations (*N*) arising under the action of BU.[2]

Fourth horizontal line: mutations (*AP*) arising under the action of AP in bacteria B-97.[3]

Fifth horizontal line: mutations (*AP*) arising under the action of AP in bacteria B-96.[3]

Mutants for which no recombination has been found are stacked at the same site of the drawing, all others are horizontally displaced. This map is identical with the one given in an earlier publi-

mutants. While most of the spontaneous mutants did not show any increase in revertants with either AP or BD, most of the mutants induced by base analogues could also be induced to revert by one or both of the analogues. This mutual exclusion must correspond to a fundamental difference in the mutagenic mechanism.



cation[3] but this time the shading of each box indicates the reversion inducibility by AP and BD found in *spot tests:*

- ⊠ = Nonreverting.
- ⊡ = Reverts spontaneously, but reversion is not induced by either AP or BD.
- ▨ = Reverts spontaneously, reversion is strongly inducible by AP and weakly by BD.
- ▨ = Reverts spontaneously, reversion is strongly inducible by BD and weakly by AP.
- ■ = Reverts spontaneously, reversion is about equally inducible by AP and BD.
- ▨ = Reverts spontaneously, reversion is inducible by AP while no BD induction could be observed in spot tests.
- ⬚ = Reverts spontaneously. Induction of reversions has not been tested.

Some exceptions to the general rule are found among the spontaneous mutants, showing that some spontaneous mutations are produced by a mechanism akin to that responsible for the induction of mutations by our base analogues. One of the exceptions is mutant *r* 215 which was included in Table 1 of liquid culture tests.

TABLE 2

SUMMARY OF THE SPOT TESTS

Reverting Mutants Which Arose in the Presence of	Total Number	Inducible		Noninducible	
		Number	%	Number	%
BD + 4× thymidine	100	80	80	20	20
BU	64	61	95	3	5
AP	98	96	98	2	2
Spontaneously	110	15	14	95	86
Proflavine	55	1	2	54	98

All untested spontaneous, proflavine, and BU induced mutants are here included which have the same genetic location and spontaneous reversion index as a mutant tested for reversion induction. They are assumed to be reversion inducible or noninducible according to the mutant tested.

A small number of the *N*- and *AP*-mutants, which arose in the presence of BU (or BD) and AP, respectively, could not be induced to revert by either AP or BD. All of them may belong to the small background of spontaneous mutants among the induced ones. This becomes especially clear when those *N*-mutants are inspected which arose under the action of 50 μg/ml BD and 200 μg/ml thymidine (second horizontal line in Fig. 1). In this case the comparison of the mutagenic effect with and without BD had shown[3] that 10 to 20 per cent of these mutants must be of spontaneous origin. Several of these mutants had been found, indeed, to coincide genetically with some of the spontaneous hot spots; this agrees entirely with the induction test for revertants, since all these mutants could not be induced to revert, and therefore behave like spontaneous mutants even in this respect. (This observation also shows that probably most or all nontested mutants of a particular genetic location behave with respect to reversion induction, like the one mutant tested provided they have the same spontaneous reversion index.)

No mutant could be induced to revert which cannot revert spontaneously.

Mutants induced by BU tend to be much more inducible to revert by AP than by BD, and mutants induced by AP tend to be more inducible by BD than by AP.

Table 2 contains also the results of spot tests for proflavine induced mutants (mutants of Brenner, Benzer, and Barnett[4]). Forty genetically different mutants were tested and only one, a single occurrence, was inducible to revert by AP and BD.

Further Examination of Revertants.—A plaque appearing when phages of an *r*II-type stock are plated with bacteria K can be due to four different reasons:

(1) A "back mutation" to the standard genotype of the *r*II region.

(2) A "partial reversion" that is a mutation within the *r*II region to *another*, fully or partially functional, genotype.

(3) A "suppressor mutation" at another than the *r*II locus.

(4) Leakiness, i.e., ability of the mutants to grow slightly on K so that there is a chance for a plaque to form. Such leaky mutants have not been examined here.

We want to show that most of the *r*II-type mutants can revert only by back mutations; some can, in addition, undergo a partial reversion, while suppressor mutations do not occur.

As a first examination, several plaques on K are picked and replated on B and K. 55 mutants were examined in this way (21 of them noninducible, 34 inducible; most belonging to different genetic sites). Of the inducible mutants, both spontaneous and induced revertants were analyzed. The results are given in Table 3. It is apparent that *most mutants* give rise to revertants of standard phenotype

TABLE 3

SUMMARY OF THE EXAMINATION OF SPONTANEOUS AND INDUCED REVERTANTS WITH RESPECT TO PLAQUE TYPE ON BACTERIA B AND K

Mutants Tested	Spontaneous "Revertants" on K	Isolated and Replated No.	Isolated and Replated On B	Isolated and Replated On K	Reversions Induced by AP No.	Reversions Induced by AP On B	Reversions Induced by AP On K	Revertants Replated BD No.	Revertants Replated BD On B	Revertants Replated BD On K
21 Base analogue noninducible	20 w	16	w	w	..	..	..	..	..	..
		4	r, w	w						
(17*r*, 4*N*)	1 mixed	=	r, w	ti, w	..	..	..	..	..	..
34 Base analogue inducible	26w	25	w	w	24	w	w	20	w	w
		1	r	w	=	r	w	=	r	w
(8*r*, 14*N*, 12*AP*)	5 w and ti	=	w	w	5	w	w	3	w	w
	5 w and ti	=	r, ti	ti	..	..	..	..	..	..
	3 mixed	=	r, w	ti, w	1	w	w	..	..	..
					1	r, w	w, ti	1	r, w	w, ti

The mutants are divided into base analogue noninducible and inducible ones (two horizontal parts). For the inducible mutants the result of spontaneous, AP, and BD induced revertants are given separately; they show that both spontaneous and induced revertants are of the same phenotype. Plaques on K are called "mixed" when their sizes vary between tiny (ti) and wild (w) and a clear distinction of two types was not possible. In contrast, for 5 base analogue inducible mutants such a distinction was possible and only revertants of the wild type were found inducible (this class includes mutants of site *N* 34).

with respect to plating on both B and K. Induction increases the proportion of these revertants and does not produce any new kind.

Sometimes even a back mutation may not restore phages of the standard phenotype, if the *r*II-type mutant was in reality a double mutant. These cases are rare, since none of our mutants carry two observable mutations in the *r*II region or an additional mutation at the *r*I locus; undetected double mutants may be of the type *r*II *r*III or *r*II minute.

A small number of induced mutants (e.g., mutants at genetic site *N* 34) yield some wild type plaques and about 100 times more tiny plaques on K. Isolating and replating the content of the wild type plaques gives again wild type plaques on B and K, while the content of tiny plaques gives *r* or tiny plaques on B and tiny plaques on K.

Also some other mutants seem to give rise to the two kinds of plaques on K. But they vary more in size between tiny and wild type, and cannot be easily classified. This includes the mutants of hot spot AP 12 and AP 133, which are contained in Table 3; 5 more mutants of site AP 12 were tested with the same result. The revertants of one plaque either produce all wild type plaques on both B and K, or all *r*-(or large wild) type plaques on B and wild type (or tiny) plaques on K. The partial reversion may in this case be nearly as efficient (on K but not on B) as the standard genotype.

In order to decide whether revertants are due to mutations in the *r*II region or to suppressor mutations, 20 revertants were backcrossed to standard type phages (all of them had standard phenotype, 5 arose spontaneously, 6 induced by BD, and 9 induced by AP). In all cases, the frequency of *r*-type phages in the progeny of a cross (mutiplicity 4 each) was not larger than that expected from the frequency of spontaneous *r* mutants present in the stocks used. The numbers were such that the reverse mutation must have occurred within the *r*II region, less than 0.2 per cent recombination units distant from the original mutation. (NOTE. The extent of the *r*II region is about 8 per cent recombination units.)

Suppressor mutations are very unlikely also in all the other cases; for, several *r*II mutants have never been observed to revert (no revertant in at least 10^{10} phages), spontaneously or by induction; genetic evidence shows that in some of these mutants the mutation cannot extend over more than a very short region of the genome.

A last argument comes from our results about induction of reversion. Suppressor mutations being excluded, we expect that each *r*II-type mutant which can revert

at all can "back mutate," spontaneously or at least under some of the mutagenic treatments. Whether it can also partially revert by another mutation depends on the particular site and mutation in question. If these partial reversions at other sites, or at the same site by another mutation, could occur for most, or all, *r*II-type mutants, it would be difficult to understand why these mutations cannot be induced by base analogues for many of the spontaneous and proflavine induced mutants. (Note that the noninducible mutants do not constitute only one class of higher specificity since they comprise spontaneously high and low reverting mutants, hot spot and nonhot-spot mutants.)

We therefore conclude that most *r*II-type mutants revert, in detectable frequency, only by back mutations.

DISCUSSION

The difference between reverting mutants inducible and noninducible by our base analogues is so drastic that it seems to reveal two kinds of fundamentally different mutagenic effects: (1) a mutagenic effect by which our base analogues induce forward mutations and their reversions, and (2) a mutagenic effect which is responsible for the formation and reversion of those mutants which cannot be induced to revert by our base analogues. Proflavine induces mutations of the second kind. Since about 10 per cent of the spontaneous mutants can be induced to revert and about 90 per cent cannot, we assume that 10 per cent of the spontaneous mutations are of the first kind, and about 90 per cent of the second. Both base analogue inducible and noninducible mutants can therefore revert spontaneously. The existence of these two spontaneous mutagenic effects is consistent with the properties of mutant *N* 34 and others of its kind (Table 1).

The two different mutagenic effects must correspond to two fundamentally different changes in DNA. It is possible to arrive at a simple molecular explanation of these changes if we assume that each *new* chain of replicating DNA is formed along a preexisting complementary chain (e.g., according to the scheme of Watson and Crick[6]) and that all mutations with which we deal in this paper are due to mistakes in DNA replications.

(1) The *mutagenic effect of the first kind*, i.e., the induction of mutations by BU and AP probably arises by mistakes in base pairing when, or after, a base analogue is incorporated into DNA.[3] Whatever the exact mechanism, a purine would always be replaced by another purine, and a pyrimidine by another pyrimidine. Hence the only possible transitions a nucleotide pair can finally undergo are

$$\begin{matrix} A \\ T \end{matrix} \longleftrightarrow \begin{matrix} G \\ H \end{matrix} = \text{mutagenic effect of the first kind} \qquad (1)$$

One would expect that for most base analogue induced mutants, the change of just one nucleotide pair is responsible for the mutant phenotype. This agrees with the fact that all induced mutants are well localized in the genome, and only 4 out of 300 do not revert.

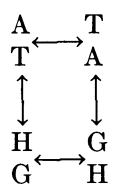
It has been discussed[3] that each base analogue can induce the transitions (1) in both directions (from A–T into G–H and vice versa). This explains why most base analogue induced mutants can also be induced to revert. In most cases the

only inducible transition seems to be that of a back mutation to the standard genotype.

A given base analogue may produce changes in one direction (1) more often than in the other. This may explain why BU induced mutants tend to be more reversion inducible by AP than by BD, and vice versa for the AP induced mutants. The preferred mutagenic direction of each base analogue is still unknown.

(2) The *mutagenic effect of the second kind* must involve molecular transitions different from (1). Even the possibility of two or more neighbouring nucleotide pairs being changed by the mechanism (1) does not seem to explain the data, since base analogue noninducible mutants readily revert spontaneously. One would have to assume di- or poly-nucleotides in the medium inducing both forward mutations *and* reversions.

A simple explanation, however, can be obtained when one assumes that the mutagenic effect of the second kind involves the "transversion" of a nucleotide pair, in which a purine is replaced by a pyrimidine and vice versa. Each (revertable) mutation would correspond to the change of one nucleotide pair only and the possible final changes are sketched in (2).



= mutagenic effect of the second kind (2)

Such a transversion may occur (one at a time) in replicating DNA in a number of ways. Most plausible for spontaneous mutations is the mistake incorporation of a purine in place of a pyrimidine or vice versa. Figure 2 shows schematically how such a mistake leads to the transversion of a nucleotide pair which subsequently replicates as such, indefinitely. The assumed purine-purine or pyrimidine-pyrimidine base pairs do not exactly fit into the Watson-Crick structure of DNA, and their presence should cause a minute distortion of the double helix. But mutational changes are very rare, and both the pairing by one or two hydrogen bonds and the rare incorporation of noncomplementary bases are structurally feasible.

It is not possible at present to decide which of the conceivable base pairings are most frequently incorporated, although the pairs A–G and T–H seem to have a higher chance, for spontaneous mutations, than pairings of identical bases. In some cases, incorporation of the wrong base may bend the end of the growing chain sufficiently off the growing direction so that further replication is difficult or impossible. This in turn may invite further mistakes leading to nonreverting mutants and larger alterations observed for some spontaneous mutants.

This picture of two mutually exclusive mutagenic effects has a number of testable consequences.

(1) All mutagenic agents which, by any conceivable mechanism, convert one purine of DNA into another purine, or one pyrimidine into another pyrimidine, can only induce mutations of the first kind. These mutations can be induced to revert by the same or, sometimes more efficiently, by another agent of this class.

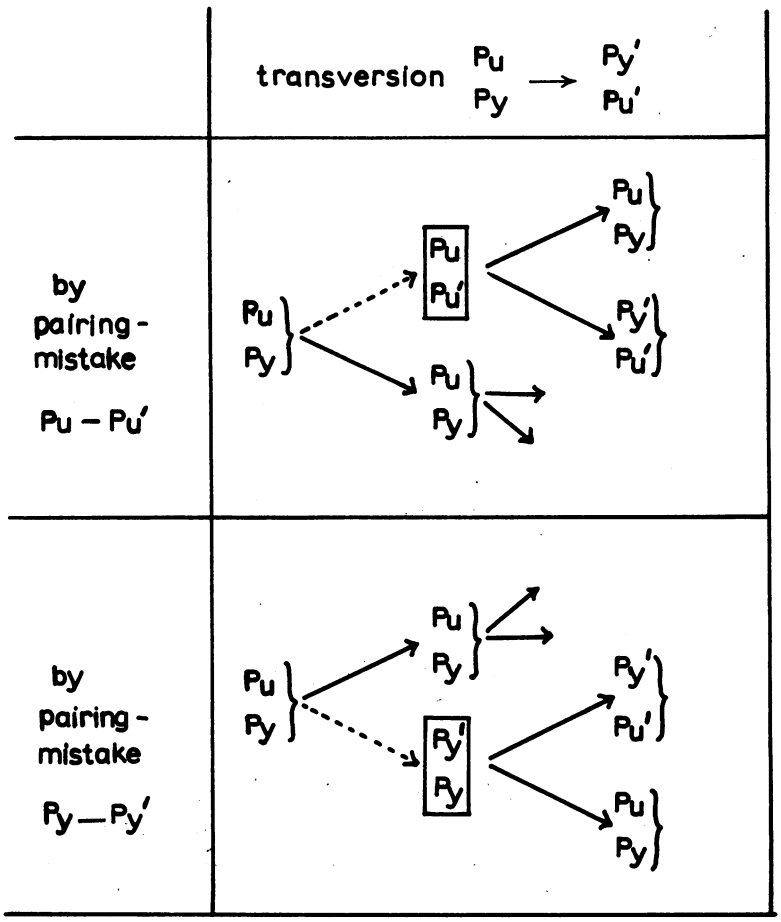


FIG. 2.—The sequence of events which may lead to the spontaneous "transversion" of a nucleotide pair. During DNA replication rarely a purine may pair with a purine- or a pyrimidine with a pyrimidine- (DNA precursor), and the wrong base pair may get incorporated into DNA. In the next DNA replication each base attaches to it its normal complementary base and thereby finalizes the purine-pyrimidine exchange. Pu = purine, Py = pyrimidine.

(2) The incorporation of a base analogue into DNA should not be able to increase the frequency of transversions, since apparently no base analogue would make the necessary pairing mistakes more often than any of the normal bases. Certain pairing mistakes of the second kind may, however, be increased, e.g. by (*a*) a large unbalance of the nucleotide ratio at the direct DNA precursor level (e.g., by thymine starvation); (*b*) agents which separate hydrogen bonds of DNA (e.g., higher temperature) or which otherwise tend to pull or keep DNA chains apart; (*c*) agents which attach (e.g., at the bases) to one nucleotide in DNA and one non-complementary nucleotide in the medium and provide a bridge of the right distance between their sugar-phosphates to enable incorporation.

An agent of kind (*b*) or (*c*) may be proflavine (= 3,6- diamino-acridine) which carries two amino groups in about 10 Å distance. One cannot decide, at present,

which of the possible attachments to nucleotides are responsible for its mutagenic effect. But this effect, apparently, is of the second kind, since proflavine induced mutants could not be induced to revert by our base analogues, although they revert spontaneously.

(3) For other organisms the same two kinds of mutagenic effects should exist. Their relative rate among the "spontaneous" mutations cannot be predicted, since it depends on the natural formation of mutagens in different organisms.

(4) The results are of obvious importance for the coding of proteins by DNA. It should be possible to group all amino acids into different classes, so that mutations of one kind can only *intra*convert amino acids of the same class, while *inter*conversions of different classes are only possible by mutations of the other kind.

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[1] Abbreviations: 2-aminopurine = AP, 5-bromouracil = BU, 5-bromodeoxyuridine = BD, adenine = A, guanine = G, thymine = T 5-hydroxymethyl cytosine = H.

[2] Benzer, S., and E. Freese, these PROCEEDINGS, **44**, 112 (1958).

[3] Freese, E., *J. Mol. Biol.*, 1959 (in press).

[4] Brenner, S., S. Benzer, and L. Barnett, *Nature*, **182**, 983 (1958).

[5] Iyer, V. N., and W. Szybalski, *J. Applied Microbiology*, **6**, 24 (1958).

[6] Watson, J. D., and F. H. Crick, *Cold Spring Harbor Symposia Quant. Biol.*, **18**, 23 (1953).

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*SOME EXAMPLES ON n-DIMENSIONAL STRUCTURAL STABILITY**

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Let $X \equiv (X_1, \ldots, X_n)$, $n \geqq 2$, be a differential system

$$\dot{x}_i = dx_i/dt = X_i(x_1, \ldots, x_n), \qquad i = 1, \ldots, n, \tag{1}$$

of class C^1 defined in the unit ball B^n, $x_1^2 + \ldots + x_n^2 \leqq 1$.

DEFINITION. The system X is said to be structurally stable in B^n if

(i) the vector field of X has no contact with the boundary S^{n-1} of B^n and, say, always points inwards;

(ii) there exists $\delta > 0$ such that, whenever a system $Y \equiv (Y_1, \ldots, Y_n)$ satisfies

$$\rho(X, Y) = \max_{B^n}\left(\sum_{i=1}^{n} |X_i - Y_i| + \sum_{i,j=1}^{n} |\partial X_i/\partial x_j - \partial Y_i/\partial x_j|\right) < \delta), \tag{2}$$

we can find a homeomorphism T of B^n onto itself mapping trajectories of X onto trajectories of Y.

This concept was introduced under a more restrictive form for the case $n = 2$ by Andronov and Pontrjagin:[1] they require that the homeomorphism T can be made arbitrarily small (ϵ-homeomorphism). In a forthcoming paper,[2] we did show that (for $n = 2$) this last definition is actually equivalent to the one we