Proc. Nat. Acad. Sci. USA
Vol. 72, No. 3, pp. 979–983, March 1975

Detection of Carcinogens as Mutagens: Bacterial Tester Strains with R Factor Plasmids

(error-prone recombinational repair/7,12-dimethylbenzanthracene/aflatoxin/nitrofurans)

JOYCE McCANN, NEIL E. SPINGARN, JOAN KOBORI, AND BRUCE N. AMES

Biochemistry Department, University of California, Berkeley, Calif. 94720

Contributed by Bruce N. Ames, December 23, 1974

ABSTRACT **We described previously a simple test on petri plates for detecting chemical carcinogens as mutagens, using an especially sensitive set of bacterial strains to detect mutagenic activity and a mammalian liver extract for carcinogen activation. We now extend the utility of the method by introducing two new bacterial strains which can detect with great sensitivity many carcinogens which we did not detect before or detected with less sensitivity. Among these carcinogens are aflatoxin B_1, sterigmatocystin, benzyl chloride, benzo[*a*]pyrene, 7,12-dimethylbenzanthracene, 1′-acetoxysafrole, and the nitrofuran food additive furylfuramide (AF-2). The new strains TA100 and TA98 contain an R factor plasmid, pKM101, in our standard tester strains TA1535 and TA1538. The R factor increases mutagenesis with certain mutagens, but not others. We present evidence that the mutagens that become more effective work through an error-prone recombinational repair.**

We have previously described a very sensitive and simple bacterial test for detecting chemical mutagens (1–4). The compounds are tested on petri plates with specially constructed mutants of *Salmonella typhimurium* as tester strains. Four tester strains have been selected, after screening hundreds of mutants, for sensitivity and specificity in being reverted from a histidine requirement back to prototrophy by a variety of mutagens. One strain (TA1535) can be used to detect mutagens causing base-pair substitutions and three (TA1536, TA1537, and TA1538) to detect various kinds of frameshift mutagens. In addition to the histidine mutation, we have added to the tester strains two additional mutations that greatly increase their sensitivity to mutagens: one causes loss of the excision repair system and the other loss of the lipopolysaccharide barrier that coats the surface of the bacteria (3).

We have shown that by adding a microsomal activation system from rat (or human) liver to the petri plates, a wide variety of carcinogens can be activated to mutagens and detected easily. Thus, an important aspect of mammalian metabolism can be duplicated in an *in vitro* test. A large group of carcinogens—aflatoxin B_1, benzo[*a*]pyrene, 2-acetylaminofluorene, etc., have been detected as reactive frameshift mutagens after liver activation (4). Each activated molecule contains a ring system capable of stacking interaction with DNA and an electrophilic group that can react with DNA (5–8). Other groups of carcinogens have been detected as mutagens causing base-pair substitutions: β-propiolactone, propanesultone, etc. (1–4). Some carcinogens, such as nitroquinoline-1-oxide (NQNO), cause both types of mutations (3).

We report here the development of two new bacterial strains which contain an R factor (plasmids carrying antibiotic resistance genes) and which greatly extend the usefulness of the test system. This work stemmed from the observation of MacPhee (9) that methyl methanesulfonate (MMS) and trimethyl phosphate were more effective in reverting *hisG46* (the histidine mutation in TA1535) when another R factor, R-Utrecht, was present. Other reports had also indicated that certain plasmids increased ultraviolet- (UV) induced mutation rates (10–12).

MATERIALS AND METHODS

Chemicals were obtained as follows: Ampicillin trihydrate (Bristol Labs); methyl methanesulfonate (MMS), bis(2-chloroethyl)amine·HCl, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), sterigmatocystin, benzo[*a*]pyrene, benzyl chloride, and dimethylcarbamyl chloride (Aldrich); NQNO, and 2-aminoanthracene (Schuchardt/Munich); captan (Analabs); diethyl sulfate (Fisher); aflatoxin B_1 (Calbiochem); mitomycin C, ethyl methanesulfonate, and 7,12-dimethylbenz[*a*]anthracene (Sigma); trimethyl phosphate (British Drug Houses, Ltd.); furylfuramide [2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide: trade name AF-2] (gift of T. Sugimura: National Cancer Research Institute, Tokyo); 1′-acetoxysafrole, 1-phenyl-1-(3,4-xylyl)-2-propynyl-*N*-cyclohexyl carbamate, and 2-nitrosofluorene (gifts of J. and E. Miller); ICR-191 and ICR-191-OH (7, 8) (gifts of R. Peck and H. J. Creech); niridazole (gift of E. Bueding); dimethylsulfoxide (spectrophotometric grade) (Schwarz/Mann).

Bacterial Strains, some previously described (1, 3), are *Salmonella typhimurium* unless noted.

R Factor Transfer followed the procedure of K. Mortelmans and B. A. D. Stocker (12). Fully grown unshaken nutrient broth cultures of the R-factor-containing donor strain (0.1 ml) and the recipient strain (1.0 ml) were diluted in nutrient broth (9 ml) and incubated for 20 hr at 37° without shaking. Ampicillin-resistant recipients were selected on minimal-glucose petri plates containing ampicillin (0.8 mg per plate), and required supplements (usually histidine and biotin). Donor strains were SL1156 (*trpD1*/R-Utrecht), SL1127 (*pur pro*/R46), and TA2000 (*purF145*/pKM101) which we constructed by transferring pKM101 from *hisG46*/pKM101 (SL3379; = TA92) to *purF145*. SL strains were kindly provided by B. A. D. Stocker.

Abbreviations: MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; NQNO, 4-nitroquinoline-1-oxide; UV, ultraviolet.

TABLE 1. *Reversion of TA100, TA98, and the standard tester strains with various mutagens and carcinogens*

Mutagen	Revertant colonies/plate*				
	μg	TA1535	TA100	TA1538	TA98
(*Spontaneous*)	—	*11*	*160*	*25*	*39*
MMS	570	5	3,244	0	5
NQNO	0.5	118	7,640	339	641
MNNG	2	1,511	18,701	0	22
Furylfuramide (AF-2)	0.02	0	1,674	0	169
Niridazole	0.2	3	1,636	180	468
Benzyl chloride	2,000	12	230	0	20
1′-Acetoxysafrole	50	7	556	46	57
1-Phenyl-1-(3,4-xylyl)-2-propynyl-*N*-cyclohexylcarbamate	100	5	2,087	0	589
Captan	10	150	820	30	184
Mitomycin C	1	0	100‡	0	—
Aflatoxin B_1†	0.1	0	2,260	80	1,940
Sterigmatocystin†	0.1	2	282	4	144
7,12-Dimethylbenz[*a*]anthracene†	20	0	1,458	72	714
Benzo[*a*]pyrene†	5	7	2,398	196	685
ICR-191	5	13	773	449	527
ICR-191-OH	100	0	0	44	18
2-Nitrosofluorene	0.5	0	462	3,936	3,841
2-Aminoanthracene†	10	333	8,835	7,725	6,801
Diethyl sulfate	5,000	14,762	2,123	0	9
Dimethylcarbamyl chloride	5,300	1,547	1,623	7	81
Bis(2-chloroethyl)-amine·HCl	50	2,708	2,306	0	0
Ethyl methanesulfonate	5,000	220	406	2	13

* Results are from linear dose–response curves after subtracting spontaneous revertants. Dose–response curves were nonlinear for diethyl sulfate, MNNG, and ethyl methanesulfonate.

† Activated by S-9 liver homogenate (4) from arochlor-induced (30) rats; one-third S-9 was used in the S-9 mix for aflatoxin, sterigmatocystin, and benzo[*a*]pyrene.

‡ TA100 was replaced by *hisG46*/pKM101 for mitomycin C (see *text*).

Storage of R Factor and Standard Tester Strains is at −80° after freezing a fresh nutrient broth culture (0.8 ml) with dimethylsulfoxide (0.07 ml) in small screw-capped vials on dry ice. Fresh cultures for mutagenesis testing are obtained by scraping a sterile wooden applicator stick over the surface of the frozen culture, inoculating nutrient broth (5 ml), and shaking overnight at 37°. The fresh culture can be kept in the refrigerator for a few days.

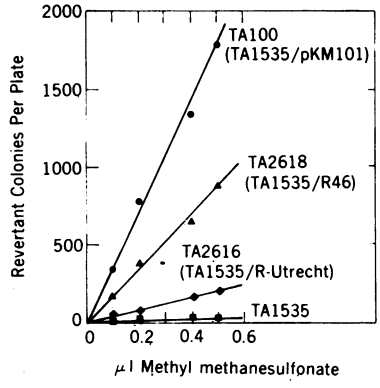

FIG. 1. Effect of R factors R-Utrecht, R46, and pKM101 on reversion of the standard tester strain TA1535 with MMS. Reversion was determined on petri plates, incorporating mutagen and bacteria directly into the top agar, as previously described (3). Revertants were scored after incubation of the petri plates for 48 hr at 37°. Spontaneous revertants have been subtracted.

The new tester strains should be checked routinely, as R factors can be lost from bacteria. We recommend that this be done before freezing by confirming ampicillin resistance and increased mutagenesis (with aflatoxin B_1 and/or MMS) compared to TA1535 and TA1538 (see Table 1). We also routinely include such positive mutagenesis controls when using these strains.

RESULTS

Effect of R Factors on Mutagenesis. In MacPhee's original experiments (9) he put an R factor plasmid, R-Utrecht (= R205) into *hisG46* and observed a 2- to 3-fold increase, compared to *hisG46* alone, in the reversion of this histidine mutation by the mutagen MMS. Mortelmans and Stocker (12) have examined a number of R factors, among which were R-Utrecht, R46, and five derivatives of R46 that lack some of the original antibiotic resistance markers, for their effect in causing an increase in UV resistance and spontaneous and UV-induced reversion rates of *hisG46*, and they have kindly given us these strains. We have found that *hisG46* containing pKM101, a derivative of R46 with only the ampicillin resistance marker, is the most sensitive to MMS-induced reversion. We have also put pKM101, R46, and R-Utrecht into our tester strain TA1535, a derivative of *hisG46* which also lacks excision repair and the lipopolysaccharide barrier. Fig. 1 shows that TA100 (TA1535 with pKM101) is the most sensitive to MMS-induced reversion. A different plasmid, pSC101 (13), was inactive.

TABLE 2. *The effect of the R factor pKM101 on reversion of hisG46 with different repair capacities*

		Revertant colonies*							
		hisG46		*recA*⁻		*uvrB*⁻		*pol*⁻	
Mutagen	μg	−R	+R	−R	+R	−R	+R	−R	+R
(*Spontaneous*)	—	*3*	*49*	*4*	*5*	*12*	*226*	*5*	*92*
MMS	2,300	32	2,007	0	2	6	1,627	2	171
NQNO	10	5	132	0	4	17	2,029	0	75
Bis(2-chloroethyl)amine	100	43	94	34	21	2,952	3,746	17	15
Furylfuramide	1	3	438	1	3				
Niridazole	10	4	155	3	3				
Trimethyl phosphate	10^4	4	112	2	3				

* Revertants per plate after subtraction of the spontaneous revertant background. Paper discs (6 mm diameter) containing mutagens were applied to petri dishes with bacteria (10^8) and a trace of histidine in a thin overlay of agar (3). Histidine revertants were scored after 48 hr incubation at 37°. The strains are: *hisG46*, *hisG46*/pKM101, TA1950 (*hisG46 uvrB*), TA2410 (TA1950/pKM101), TA2322 (*hisG46 pol-2 ara-9*), TA2644 (TA2322/pKM101), TS24 (*hisG46 recA*) (from D. Straus), and TA2411 (TS24/pKM101). We thank H. Whitfield (23) for the *pol* strain AA3011, the parent of TA2322.

Effect of Various Mutagens on the Reversion of TA100 Compared to TA1535. We have surveyed a number of mutagens for their ability to revert the *hisG46* mutation in TA100 and TA1535 (Table 1). The mutagens fall into two classes. Some mutagens, such as ethyl methanesulfonate, bis(2-chloroethyl)-amine and dimethylcarbamyl chloride, are about equally effective on the two strains (or in the case of diethyl sulfate even less effective on TA100), while others, such as the carcinogens benzyl chloride, NQNO, aflatoxin B_1, and the food additive furylfuramide, are enormously more effective on the new strain, TA100, which contains the R factor. Many of the reactive frameshift mutagens tested revert TA100 as well or better than they revert the standard frameshift tester strains TA1537 and TA1538. Mitomycin C, a carcinogen and a DNA cross-linking agent, does not revert TA100 (or *hisG46*) but does revert *hisG46*/pKM101 which has excision repair. Excision repair is required for mutagenesis with mitomycin C in *Escherichia coli* (14), and it has been suggested that mutation can only be detected if potentially lethal DNA crosslinks are broken by the excision repair system (14, 15).

Effect of pKM101 on the Frameshift Tester Strain TA1538. The tester strain TA1538 contains a well-characterized (16) histidine frameshift mutation and is reverted by a wide variety of aromatic carcinogens which can cause frameshift mutations. It is not reverted appreciably by simple alkylating agents which cause base-pair substitutions (2–4, 6). We have constructed the new strain TA98 by putting the R factor pKM101 into TA1538 and have examined a variety of mutagens (Table 1). Mutagens that revert TA1538 and TA98 fall into two classes: those that revert TA98 much better, such as aflatoxin, 7,12-dimethylbenzanthracene, and benzo-[*a*]pyrene, and those that do not, such as 2-aminoanthracene, 2-nitrosofluorene, and ICR-191.

Optimizing the Tester Strain: Other Test Mutations. We have attempted to construct a strain more sensitive than TA100 by putting the R factor pKM101 into many different histidine-requiring mutants. The mutations tested were (the numbers in parentheses are the corresponding strains containing pKM101): the missense mutations *hisG52*(TA2399), *hisG499* (TA2398), *hisD78*(TA2628), *hisD1714*(TA2404), *hisC201* (TA2620), *hisC210*(TA2621), *hisC367*(TA2622), *hisC496* (TA2395), *hisC899*(TA2623); the amber mutations *hisC31* (TA2630), *hisC50*(TA2624), *hisC121*(TA2625), *hisC340*(TA 2626); the ochre mutations: *hisC117*(TA2403), *hisC354* (TA2396), *hisC502*(TA2627), *hisC514*(TA2629), *hisO1242 hisG2101*(TA2400); the UGA mutation *hisG200*(TA2397); and the unclassified mutations *his-1743*(TA2401) and *his-1768*(TA2402) (17, 18). These strains were tested for spontaneous, MMS-, and furylfuramide-induced reversion. None of the strains was superior to *hisG46*/pKM101. We have also done considerable work with the widely used tryptophan auxotroph *E. coli* B/r WP2 and its UV-sensitive derivative WP2 *hcr*, recently used by several groups for detecting mutagenicity of nitrofurans (19–21), a class of carcinogens that does not appreciably revert our standard set of tester strains (21). We first made and compared the *gal uvrB* deletion of WP2, TA85, with the WP2 *hcr* strain used by these other groups and found the deletion superior as a tester strain for reversion by the nitrofuran furylfuramide. We then put the pKM101 plasmid into TA85, to make the strain TA93. TA93 is 50 times more sensitive to furylfuramide reversion than the original TA85, and somewhat more sensitive than TA100, but because of its slow growth and the poor response of TA93 and its deep rough derivative to aflatoxin we have preferred TA100 as a general tester strain.

Effect of pKM101 on Reversion of hisG46 with Different Repair Capacities. MacPhee and Mortelmans have previously shown that increased UV mutagenesis and protection against UV killing by R-Utrecht and R46 do not occur in *hisG46* containing a *recA* mutation (12, 22). Table 2 shows that mutagens which cause enhanced reversion (Table 1) of *hisG46* strains containing the R factor (e.g., MMS, NQNO, furylfuramide) do not revert the *recA* strains. In contrast bis(2-chloroethyl)amine, which does not show significant enhanced mutagenesis (Table 1) in the R factor strains, is mostly *rec*-independent.

We have also looked at MNNG, diethyl sulfate, and ethyl methanesulfonate and the results are generally consistent with those in Table 2, but the interpretation is complicated by nonlinear dose–response curves (Table 1).

Table 2 also shows that *uvrB* and *pol* mutations do not abolish R factor enhancement of mutagenesis, although *pol* does appear to decrease the overall level of mutagenesis. The increase in reversion by certain mutagens in *uvrB* mutants

has been previously reported (1, 3). Mortelmans and Stocker (12) have observed that R46 and pKM101 cause an increase in the number of spontaneous revertants of *hisG46* and this is shown for pKM101 in Table 2, along with a comparable increase in the *uvrB* and *pol-2* strains, but not in the *recA* strain.

DISCUSSION

We have previously described four histidine-requiring *Salmonella* tester strains for detecting carcinogens by means of their mutagenic activity (1–4). We describe here two new tester strains, TA100 and TA98, constructed by the addition of an R factor plasmid to the standard tester strains TA1535 and TA1538, and show that the new strains greatly increase the sensitivity of the test and the number of carcinogens detected. Among the many carcinogens previously detected as mutagens we show here that aflatoxin B_1, sterigmatocystin, benzo[*a*]pyrene, 7,12-dimethylbenzanthracene, and NQNO are much more easily detected with the new strains. The new strains can also detect a variety of carcinogens that do not appreciably revert our standard tester strains, such as 1′-acetoxysafrole, acetylenic carbamate derivatives, and furylfuramide (AF-2, a nitrofuran food additive that was widely used in Japan until recently). The carcinogenic nitrofurans had not previously reverted our tester set but were detected by the *E. coli* B/r strain WP2 (19–21). The new strain TA100, is considerably more sensitive than WP2 *hcr* for those nitrofurans that we have tested. Vinyl chloride is mutagenic in our system (24, 25) and we have shown the utility of TA100 in detecting chloroacetaldehyde, a possible active metabolic product of vinyl chloride. (J. McCann, V. Simmon, and B. N. Ames, in preparation).

The mechanism whereby the R factor enhances mutagenesis is not fully understood at present; however, recombinational repair does appear to be involved, as indicated by the following four points: (*1*) It is known that certain mutagens cause damage to the DNA that is not mutagenic directly, but that the mutations are caused by errors introduced when the damage is repaired by error-prone recombinational repair. This was first shown many years ago by Witkin, who found that UV light is not mutagenic in bacteria with a *rec* mutation, even though they are much more sensitive to killing by UV. This has also been shown for a variety of chemical mutagens, such as MMS and NQNO, by Kondo (14), and Table 2 shows the *recA* dependence of these, furylfuramide, niridazole, and trimethyl phosphate. (*2*) With all of these chemical mutagens the R factor causes a marked increase in mutagenesis (Tables 1 and 2). MacPhee (9, 11) has shown this with R-Utrecht for UV, MMS, and trimethyl phosphate. (*3*) The effect of the R factor on reversion of the *hisG46* mutation by these chemical mutagens (Table 2), by UV (22), or spontaneously, cannot be detected in a strain with a *recA* mutation but can when there is a *uvrB* or *pol* mutation. (*4*) Other mutagens, such as bis(2-chloroethyl)-amine or ethyl methanesulfonate, appear to be relatively independent of the *rec* system in causing mutation (Table 2; ref. 14), and these mutagens are not stimulated by the presence of the R factor (Table 1).

A postulated mechanism for R-factor-stimulated mutagenesis must then take into account *recA*-dependent repair. As recombinational repair needs a gap in one strand of the DNA to work (26), it seems likely that certain mutagenic events could lead to nicks, which then allow recombinational repair and base-pair substitutions or frameshift errors. The class of mutagens not showing an enhancement by the R factor would presumably be mutagenic directly. MacPhee (11, 22) has suggested that the role of the R factor is to enhance this error-prone repair system and has recently shown that a strain containing R-Utrecht has increased DNA polymerase activity (27). Mortelmans (12) has suggested that the R factor could supply a mistake-prone DNA polymerase. We have obtained evidence (in collaboration with D. Lackey and S. Linn) that the pKM101-containing strains have a new endonuclease which could play a role in nick and gap formation in the mutagenized DNA. At this point, however, the exact nature of the role of the R factor is not known.

Frameshift type errors are apparently caused by slippage of a repetitive sequence in the DNA, for example the C-G-C-G-C-G-C-G sequence in TA1538 (16). There is markedly increased frameshift mutagenesis by certain aromatic carcinogens, such as aflatoxin or dimethylbenzanthracene, in TA98 compared to TA1538. This may also be due to nick generation, with the additional factor of the mutagen stabilizing the mispairing by a stacking interaction with DNA (4). Frameshift mutations caused by some of the other reactive carcinogens, such as activated 2-aminoanthracene or 2-nitrosofluorene, are not increased by the presence of the R factor in TA98. Reversion by nonreactive frameshift mutagens, such as ICR-191-OH (and 9-aminoacridine on TA1537/pKM101), is also not increased by the R factor. We have not seen an effect of any repair system on nonreactive type frameshift mutagens.

Although the simple alkylating agents do not cause frameshift mutations, even in the strains carrying R factors, many of the reactive frameshift mutagens can revert the missense mutation *hisG46* (in the TA1535 tester strain) when the R factor is present, and this is presumably due to error-prone recombinational repair after DNA damage. It is still unclear, however, why some of the reactive frameshift mutagens such as activated 2-aminoanthracene and 2-nitrosofluorene are stimulated by the R factor in TA1535 but not in TA1538. Further work on the *rec* dependence of frameshift mutagens and the mechanism of frameshift mutagenesis is needed to clarify this point.

We recommend that TA98 (TA1538/pKM101) and TA100 (TA1535/pKM101) be added to the set of tester strains previously introduced. They can replace TA1535 and TA1538 for general screening for mutagenicity. Strains TA1535 and TA1538 will still be useful for classifying mutagens as to type, without the complications of the R factor, and, as they have a lower spontaneous mutation rate than TA98 and TA100, they will be more convenient and more sensitive in studying particular mutagens whose effectiveness is not increased by the R factor. One of the old frameshift tester strains, TA1536, can now be dropped from the set of tester strains, as extremely few mutagens revert it and those that do can be detected well with the other frameshift tester strains. The other frameshift tester strain in the set, TA1537, is still useful, though we are working on a somewhat more sensitive strain, TA90, with similar characteristics, that appears to have a run of 8 Cs in the DNA at the site of a +1 frameshift mutation. This strain and its pKM101 derivative (TA97) will be described in a separate communication. Another tester strain is also being developed for the detection of agents such as the

carcinogenic antibiotic mitomycin C, which crosslinks DNA and is not active in the presence of a *uvrB* mutation (Table 1; ref. 14).

The *Salmonella*/liver test system is now in use in hundreds of laboratories throughout the world and large numbers of carcinogens and noncarcinogens have been tested. It is being used to examine mutagenic metabolites in urine (28, 29) and several groups are starting to screen the human population. It is also being used to detect mutagenic activity in complex mixtures such as cigarette smoke and its fractions (30). We are in the process of compiling the results for the many hundreds of carcinogens and noncarcinogens that have been tested in the system. The preliminary results indicate that over 70% of the carcinogens tested are detected as mutagens. In almost every case the test does not respond to known noncarcinogens. Some of the carcinogens previously missed can now be shown to be good mutagens with the new strains (Table 1) and we suspect that the test system will eventually detect over 80% of chemical carcinogens as mutagens.

We are living in a sea of chemicals that have not been tested for mutagenicity and carcinogenicity. It has been estimated that 80% of human cancer is due to environmental causes (31), yet only a very small percentage of the chemicals humans are exposed to can be tested in the extremely expensive and long-term cancer tests in rodents. Practically no tests are being done on mutagenicity of environmental chemicals in mammals. Microbial tests are rapid and inexpensive, and are being used as efficient screens to detect potentially hazardous chemicals. Compounds that give a positive test for mutagenicity in microbial tests should be considered potential hazards for man and should be scrutinized for benefit, risk, and need for further testing by other more time-consuming methods. A solution to the problem of cancer and birth defects is likely to be prevention.

We are indebted to B. A. D. Stocker and K. Mortelmans for strains, unpublished information, and discussion; to D. G. MacPhee, D. S. Straus, and W. E. Durston for strains; to J. Richards and V. Paulus for testing dimethylbenzanthracene and niridazole; to Edith Yamasaki for construction of TA98 and to S. Kondo for discussion. This work was supported by Atomic Energy Commission Grant AT(04-3)34 P.A.156 to B.N.A. J.McC. was supported by a postdoctoral fellowship from the California Division of the American Cancer Society.

1. Ames, B. N. (1971) in *Chemical Mutagens: Principles and Methods for their Detection*, ed. Hollaender, A. (Plenum Press, New York), Vol. 1, pp. 267–282.
2. Ames, B. N. (1972) in *Mutagenic Effects of Environmental Contaminants*, eds. Sutton, E. & Harris, M. (Academic Press, New York), pp. 57–66.
3. Ames, B. N., Lee, F. D. & Durston, W. E. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 782–786.
4. Ames, B. N., Durston, W. E., Yamasaki, E. & Lee, F. D. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2281–2285.
5. Ames, B. N., Sims, P. & Grover, P. L. (1972) *Science* **176**, 47–49.
6. Ames, B. N., Gurney, E. G., Miller, J. A. & Bartsch, H. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3128–3132.
7. Ames, B. N. & Whitfield, H. J., Jr. (1966) *Cold Spring Harbor Symp. Quant. Biol.* **31**, 221–225.
8. Creech, H. J., Preston, R. K., Peck, R. M., O'Connell, A. P. & Ames, B. N. (1972) *J. Med. Chem.* **15**, 739–746.
9. MacPhee, D. G. (1973) *Appl. Microbiol.* **26**, 1004–1005.
10. Howarth, S. (1966) *Mutat. Res.* **3**, 129–134.
11. MacPhee, D. G. (1973) *Mutat. Res.* **18**, 367–370.
12. Mortelmans, K. (1975) PhD Dissertation, Stanford University.
13. Cohen, S. N., Chang, A. C. Y., Boyer, H. W. & Helling, R. B. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3240–3244.
14. Kondo, S., Ichikawa, H., Iwo, K. & Kato, T. (1970) *Genetics* **66**, 187–217.
15. Murayama, I. & Otsuji, N. (1973) *Mutat. Res.* **18**, 117–119.
16. Isono, K. & Yourno, J. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1612–1617.
17. Whitfield, H. J., Jr., Martin, R. G. & Ames, B. N. (1966) *J. Mol. Biol.* **21**, 335–355.
18. Hartman, P. E., Hartman, Z., Stahl, R. C. & Ames, B. N. (1971) *Advan. Genet.* **16**, 1–34.
19. Kada, T. (1973) *Jap. J. Genet.* **48**, 301–305.
20. McCalla, D. R. & Voutsinos, D. (1974) *Mutat. Res.* **26**, 3–16.
21. Yahagi, T., Nagao, M., Hara, K., Matsushima, T., Sugimura, T. & Bryan, G. T. (1974) *Cancer Res.* **34**, 2266–2273.
22. MacPhee, D. G. (1973) *Mutat. Res.* **19**, 357–359.
23. Whitfield, H. J. & Levine, G. (1973) *J. Bacteriol.* **116**, 54–58.
24. Rannug, U., Johansson, A., Ramel, C. & Wachtmeister, C. A. (1974) *Ambio* **3**, 194–197.
25. Bartsch, H., Malaveille, C. & Montesano, R. (1975) *Int. J. Cancer*, in press.
26. Cooper, P. K. & Hanawalt, P. C. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 1156–1160.
27. MacPhee, D. G. (1974) *Nature* **251**, 432–434.
28. Durston, W. E. & Ames, B. N. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 737–741.
29. Commoner, B., Vithayathil, A. J. & Henry, J. I. (1974) *Nature* **249**, 850–852.
30. Kier, L. D., Yamasaki, E. & Ames, B. N. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 4159–4163.
31. Epstein, S. S. (1974) *Cancer Res.* **34**, 2425–2435.