that reside in the gut but not to γ streptococcus that does not reside there. Diener (*10*) has interpreted the specific binding of microorganisms on the surface of lymphoid cells as an indication of production of specific antibody by these cells. Friedman *et al.* (*11*), with *E. coli*, substantitated the fact that such surface binding of bacteria was due to production of specific antibody. We conclude that soon after hatching, bursal lymphocytes are involved in recognition of antigen present in the gut and they carry out active antibody synthesis in response.

We have shown previously that immunoglobulin synthesis takes place primarily in the medulla of bursal follicles after the lymphoid cells have undergone a proliferative cycle in the follicular cortex (*3*). Other studies failed to demonstrate specific antibody formation by the bursa (*7, 8*). These investigators considered the bursa to be exclusively a site where cells can differentiate before emigrating to peripheral sites for active antibody synthesis. This view is too restrictive and must be modified to include our new data. The bursa, in addition to being a site for differentiation of antibody forming cells at a critical period in ontogenesis, begins functioning actively in the first week after hatching in the local defense system of the gut; it produces antibodies against the environmental antigens encountered by this well-developed lymphoid organ. That this is a local defense system was clearly shown by the marked increase in the production of PFC by bursae, and not by spleen, when the sheep RBC's were introduced into the bursal lumen. Further, although bursal cells reacted with *E. coli* and produced BAC, both splenic and thymic lymphocytes failed to react with this organism. We postulate that antigens reach the follicular cells by direct passage from the gut by way of the epithelium overlying the lymphoid follicles. Only when the sheep RBC's were introduced into the lumen did we detect antibody-producing cells. The epithelium associated with the follicle has the capability of pinocytosis and the necessary fine structure for this function (*12*). Therefore, only when antigens gain entrance to the follicle by way of the lumen are they capable of setting in motion a chain of events that culminates in production of specific antibodies. Thus, although unrecognized up to now, one of the major functions of the bursa, shortly after hatching of the chicken and until resorption of the organ, is related to local immunological defense against bacterial organisms present in the gut.

PIERSON J. VAN ALTEN
Department of Anatomy, University of Illinois at The Medical Center, Chicago 60680

HILAIRE J. MEUWISSEN
Kidney Disease and *Birth Defects Institutes, New York State Department of Health, Albany, 12208*

References and Notes

1. T. S. Chang, B. Glick, A. R. Winter, *Poultry Sci.* **34**, 1186 (1955); B. Glick, *ibid.* **37**, 240 (1958).
2. P. J. Van Alten, W. A. Cain, R. A. Good, M. D. Cooper, *Nature* **217**, 358 (1968); M. D. Cooper, W. A. Cain, P. J. Van Alten, R. A. Good, *Int. Arch. Allergy* **35**, 242 (1969); W. A. Cain, M. D. Cooper, P. J. Van Alten, R. A. Good, *J. Immunol.* **102**, 671 (1969); E. J. Motika and P. J. Van Alten, *ibid.* **107**, 512 (1971).
3. H. J. Meuwissen, K. Gajl-Peczalska, G. T. Kaplan, D. Frommel, T. J. Linna, R. A. Good, in preparation.
4. P. W. Kincade and M. D. Cooper, *J. Immunol.* **106**, 371 (1971).
5. G. M. Thorbecke, N. L. Warner, G. H. Hochwald, S. H. Ohanian, *Immunology* **15**, 123 (1968).
6. T. H. Kerstetter, I. O. Buss, H. A. Went, *J. Exp. Zool.* **149**, 233 (1962).
7. P. B. Dent and R. A. Good, *Nature* **207**, 491 (1965).
8. B. Glick and S. Whatley, *Poultry Sci.* **46**, 1587 (1967).
9. A. J. Cunningham and A. Szenberg, *Immunology* **14**, 599 (1968).
10. E. Diener, *J. Immunol.* **100**, 1062 (1968).
11. H. Friedman, J. Allen, M. Landy, *ibid.* **103**, 204 (1969).
12. D. E. Bockman and M. D. Cooper, *Fed. Proc.* **30**, 511 (1971).
13. This work was supported by grant AI-08289 from the National Institutes of Health. We thank Mrs. Zanet F. Gill for technical assistance, Mr. James Robinson for providing the γ streptococci, and Dr. R. A. Good for helpful discussions.

29 July 1971; revised 27 September 1971

Epoxides of Carcinogenic Polycyclic Hydrocarbons Are Frameshift Mutagens

Abstract. *K-region epoxides of the carcinogens benz*[a]*anthracene, dibenz*[a,h]*anthracene, and 7-methylbenz*[a]*anthracene are mutagenic in strains of* Salmonella typhimurium *designed to detect frameshift mutagens. Parent hydrocarbons, K-region diols and phenols and some other epoxides are inactive as mutagens in these tests. Polycyclic hydrocarbon epoxides, and other presumed proximal carcinogens, are discussed as examples of intercalating agents with reactive side chains. It has been shown previously that intercalating agents with reactive side chains are potent frameshift mutagens.*

Polycyclic planar aromatic compounds, such as the acridines, which intercalate in the DNA base pair stack (*1*) are mutagens that cause additions and deletions of bases in a gene (*2–4*). The mechanism is thought to be that the intercalation stabilizes a mispairing in the DNA during replication or repair synthesis, or during recombination, which causes the errors of addition or deletion (*3, 5*). These mutagens are called frameshift mutagens because the reading frame of the messenger RNA (mRNA) is shifted by the addition or deletion of a base, and this effect distinguishes them from the usual mutagens that cause base pair substitutions (*3*). When an intercalating agent also has a side chain attached to it that can react with DNA, it is a more potent frameshaft mutagen by one or two orders of magnitude (*4*). Polycyclic hydrocarbons, which are known to intercalate into DNA (*6*), are converted into epoxides by microsomal enzyme systems (*7*). These epoxide intermediates are chemically and biologically active compounds (*8*) that may well be involved in hydrocarbon carcinogenesis (*9*). We report here that polycyclic hydrocarbon K-region epoxides cause frameshift mutations in bacteria but that the hydrocarbons themselves and the hydroxylated derivatives are inactive. K-region epoxides are also mutagenic to T2 bacteriophage and to mammalian cells in culture, but the types of mutation produced have not been characterized (*10*).

We have used a set of tester strains (*11*) that have been developed in *Salmonella typhimurium* for detecting and classifying mutagens. We score, by a simple back mutation test, the reversion from histidine requirement to growth on minimal medium. Three of the strains (TA1531, TA1532, and TA1534) are designed for detecting frameshift mutagens of varying specificity. Each strain has a frameshift mutation in one of the genes of the histidine operon thus causing the strain to have a histidine requirement. Another strain (TA1530), used to detect mutagens that cause base pair substitutions, has a base pair change in the histidine *G* gene. All four

strains also have a defective excision repair system (a deletion through *uvrB*) which makes them much more sensitive to any mutagen that reacts with DNA to form repairable lesions (*11*).

The hydrocarbon epoxides and the K-region dihydrodiols and phenols were prepared as described (*12*) and were added, as solutions in dimethyl sulfoxide (spectrophotometric grade, Schwarz/Mann), to the top agar of test plates.

In initial qualitative experiments, the polycyclic hydrocarbons phenanthrene, benz[*a*]anthracene, 7-methylbenz[*a*]anthracene, dibenz[*a,h*]anthracene, 3-methylcholanthrene, and chrysene and their respective K-region epoxides, dihydrodiols, and phenols were tested against each of the four tester strains. The K-region epoxides of benz[*a*]anthracene and 7-methylbenz[*a*]anthracene were the only compounds that caused mutations in the strains designed to detect frameshift mutagens; none of the compounds was active against strain TA1530 that detects base-substitution mutagens.

The hydrocarbon epoxides were subsequently examined in more detail and the results (Table 1) confirm those obtained in the qualitative tests. Benz[*a*]anthracene 5,6-oxide was quite active against the frameshift tester TA1532, and 7-methylbenz[*a*]anthracene 5,6-oxide was less so. Again none of the epoxides showed mutagenic activity toward the base substitution tester strain TA1530. In all quantitative experiments proportionality was observed at lower concentrations between the amounts of epoxide and the mutagenic response, but a falling off in this response was noted at higher concentrations, possibly because of epoxide insolubility.

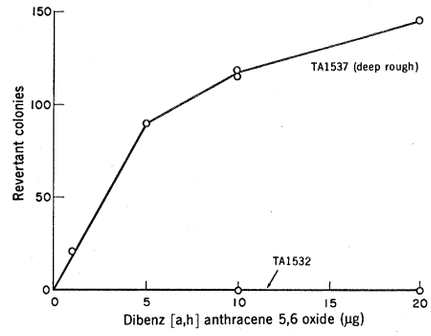

Fig. 1. The number of revertant colonies of strain TA1532 and TA1537 per petri plate as a function of the dose of dibenz[*a,h*]anthracene 5,6-oxide. The three control plates of TA1537 showed 3, 4, and 9 colonies, and the two control plates for TA1532 showed 14 and 15 colonies. The average of each set of controls has been subtracted in each experiment. The procedures were the same as in Table 1, except that no more than 20 μl of a solution of the mutagen (1 mg/ml) in dimethyl sulfoxide was added to the top agar.

A forward mutation test, with azetidinecarboxylic acid resistance (*11*), which detects both base substitutions and frameshift mutations but with a lower sensitivity than the specific back mutation tests, was also tried on a number of epoxides. Cultures of *S. typhimurium* LT-2 were grown in minimal medium (*11*) containing 20 μg of epoxide per milliliter. Benz[*a*]anthracene 5,6-oxide and 7-methylbenz[*a*]anthracene 5,6-oxide gave positive results, the rate being 8 and 11 times that of the spontaneous rate, respectively. The epoxides of phenanthrene, 3-methylcholanthrene, and dibenz[*a,h*]anthracene were not mutagenic.

Information on both the mutagenicity and the ability of hydrocarbon epoxides to cause malignant transformation of rodent cells in culture is accumulating (*9, 10*) so that attempts to correlate mutagenicity with carcinogenicity can be made. In the *Salmonella* tests this correlation is not exact because the K-region epoxides derived from the potent carcinogens dibenz[*a,h*]anthracene and 3-methylcholanthrene were not mutagenic, although both epoxides did induce malignant transformation of rodent cells in vitro (*9, 13*) and caused uncharacterized mutations in T2 bacteriophage and in mammalian cells (*10*). One explanation for this is that the barrier of the lipopolysaccharide on the bacterial outer surface prevents the passage of these two compounds (the two largest of the group) to the membrane. Deep rough mutants of *S. typhimurium* that are lacking most of the lipopolysaccharide (a component of the bacterial outer membrane) are much more sensitive to a variety of antibiotics and dyes (*14*). Consequently we have constructed (*15*) a deep rough derivative (TA1537) of tester strain TA1532 in order to investigate this aspect.

This new strain, TA1537, was found to be an excellent tester strain for detecting frameshift mutagenesis caused by dibenz[*a,h*]anthracene 5,6-oxide. The response of strain TA1537 and of the parent strain TA1532 to varying doses of this carcinogen are compared in Fig. 1. It is apparent that the deep rough mutation has made the bacterial genome accessible to the mutagen so that the frameshift mutation in the histidine operon can now be reverted. With other epoxides, strain TA1537 was similar in response to the parent strain, except that chrysene epoxide showed some activity with TA1537. Methylcholanthrene epoxide, which one might have expected to be mutagenic, was not active with either strain. One possible explanation for this is that it is too large to penetrate the remaining lipopolysaccharide.

We have also obtained indirect evidence that the epoxides are reacting with the bacterial DNA, as well as intercalating, by comparing their mutagenic effect on strains with and with-

Table 1. Revertant colonies (histidine nonrequiring) per petri plate. Each number is the result of a count of a separate plate. The boldface numbers are judged to be significantly different from the controls. The procedure was that described (*11*), except that 0.5 ml of a dimethyl sulfoxide solution of the potential mutagen was added to the top agar in all plates. The plates were incubated for 2 days at 37°C. All solutions were sterile.

Compound added	Amount (μg)	Revertant colonies (No./plate) from tester strains:			
		TA1530	TA1531	TA1532	TA1534
None (control)		23,45,45, 58,68	2,4,4,4, 5,8	10,10,15, 15,17,23	19,23,29, 29
Phenanthrene 9,10-oxide	134	65,79	4	16	20
Benz[*a*]anthracene 5,6-oxide	34			**120**	
	67			**230**	
	134	49,62	2	**275**	31
Benz[*a*]anthracene 8,9-oxide	134	39	1	12	15
	268		3	3	31
7-Methylbenz[*a*]anthracene 5,6-oxide	67			**52**	
	134	54	3	22	**50**
	196			**83**	
Dibenz[*a,h*]anthracene 5,6-oxide	134	69	1	23,25	11
3-Methylcholanthrene 11,12-oxide	134	67	3	12	25
	268			9	
Chrysene 5,6-oxide	134	48	6	6	20
	268		3		

out excision repair. Mutagens that can both intercalate and form a covalent bond with DNA, such as the quinacrine–half mustard mutagen ICR 191, are much more mutagenic in reverting a particular histidine mutation in a strain without excision repair than in a strain with repair, while simple intercalating mutagens such as quinacrine or 9-aminoacridine are equally effective on the two strains (*11*). Benz[*a*]anthracene 5,6-oxide is negative, at the level of sensitivity of our test, in reverting the *hisC3076* mutation in a *uvrB*+ strain while, as shown in Table 1, it is quite effective on the double mutant TA1532 (that is, *hisC3076 uvrB*).

A number of other carcinogenic compounds have been shown to be powerful frameshift mutagens (*11*). A series of fluorene carcinogens are frameshift mutagens and one of the carcinogenic metabolic products with a reactive group, 2-nitrosofluorene, is one of the most potent frameshift mutagens we have ever tested (*16*). The well-known carcinogen 4-nitroquinoline *N*-oxide is also a frameshift mutagen in the *Salmonella* tests (*17*).

We think it is reasonable to propose that polycyclic hydrocarbons are carcinogenic because of the mutagenicity of epoxide intermediates formed during metabolism and that the mechanism of action may involve intercalation followed by covalent reaction. This proposed mode of action involving stabilization of DNA mispairing could explain the observations of Fahmy and Fahmy (*18*) that, in *Drosophila*, polycyclic hydrocarbons preferentially cause bobbed and minute mutations, which are large deletions in regions of duplicated gene clusters for ribosomal RNA and transfer RNA.

Note added in proof: We are indebted to J. R. Roth for raising the point that the epoxide induced mutations could conceivably be due to external frameshift suppressors, one class of which might be caused by base pair substitutions (*19*). We have ruled out this possibility by analyzing 48 epoxide induced revertants of TA1532, none of which were due to external suppressors.

BRUCE N. AMES
Department of Biochemistry,
University of California,
Berkeley 94720

P. SIMS, P. L. GROVER
Chester Beatty Research Institute,
Institute of Cancer Research,
Royal Cancer Hospital,
London SW3 6JB, England

References and Notes

1. L. S. Lerman, *J. Mol. Biol.* **3**, 18 (1961); H. J. Li and D. M. Crothers, *ibid.* **39**, 461 (1969); D. O. Jordon and L. N. Sansom, *Biopolymers* **10**, 339 (1971); M. Sakoda, K. Hiromi, K. Akasaka, *ibid.*, p. 1033; G. Löber and G. Achtert, *ibid.* **8**, 595 (1969); R. L. O'Brien, J. L. Allison, F. E. Hahn, *Biochim. Biophys. Acta* **129**, 662 (1966).
2. S. Brenner, L. Barnett, F. H. C. Crick, A. Orgel, *J. Mol. Biol.* **3**, 121 (19(→ L. Barnett, S. Brenner, F. H. C. Crick, R. G. Shulman, R. J. Watts-Tobin, *Phil. Trans. Roy. Soc. London Ser. B* **252**, 487 (1967).
3. J. W. Drake, *The Molecular Basis of Mutation* (Holden-Day, San Francisco, 1970).
4. B. N. Ames and H. J. Whitfield, Jr., *Cold Spring Harbor Symp. Quant. Biol.* **31**, 221 (1966); B. N. Ames, unpublished data; H. J. Creech, R. K. Preston, R. M. Peck, A. P. O'Connell, B. N. Ames, *J. Med. Chem.*, in press.
5. G. Streisinger, Y. Okada, J. Emrich, J. Newton, A. Tsugita, E. Terzaghi, M. Inouye, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 77 (1966); J. Yourno, *J. Mol. Biol.* **62**, 223 (1971); ———, I. Ino, T. Kohno, *ibid.*, p. 233.
6. E. Boyland and B. Green, *Brit. J. Cancer* **16**, 507 (1962); A. M. Liquori, B. DeLerma, F. Ascoli, C. Botre, M. Trasciatti, *J. Mol. Biol.* **5**, 521 (1962); A. M. Craig and I. Isenberg, *Proc. Nat. Acad. Sci. U.S.* **67**, 1337 (1970).
7. J. K. Selkirk, E. Huberman, C. Heidelberger, *Biochem. Biophys. Res. Commun.* **43**, 1010 (1971); P. L. Grover, A. Hewer, P. Sims, *Fed. Eur. Biochem. Soc. Lett.* **18**, 76 (1971).
8. P. L. Grover and P. Sims, *Biochem. Pharmacol.* **19**, 2251 (1970); P. L. Grover, J. A. Forrester, P. Sims, *ibid.* **20**, 1297 (1971).
9. P. L. Grover, P. Sims, E. Huberman, H. Marquardt, T. Kuroki, C. Heidelberger, *Proc. Nat. Acad. Sci. U.S.* **68**, 1098 (1971).
10. M. J. Cookson, P. Sims, P. L. Grover, *Nature New Biol.* **234**, 186 (1971); E. Huberman, L. Aspiras, C. Heidelberger, P. L. Grover, P. Sims, *Proc. Nat. Acad. Sci. U.S.* **68**, 3195 (1971).
11. B. N. Ames, in *Chemical Mutagens: Principles and Methods for Their Detection*, A. Hollaender, Ed. (Plenum Press, New York, (1971); vol. 1, chap. 9; ———, in *Mutagenic Effects of Environmental Contaminants*, E. Sutton and M. Harris, Eds. (Academic Press, New York, in press); ———, in preparation.
12. E. Boyland and P. Sims, *Biochem. J.* **95**, 778 (1965); *ibid.* **97**, 7 (1965); P. Sims, *ibid.* **105**, 591 (1967); *ibid.* **125**, 159 (1971).
13. H. Marquardt, T. Kuroki, E. Huberman, J. Selkirk, C. Heidelberger, P. L. Grover, P. Sims, in preparation.
14. R. J. Roantree, T. Kuo, D. G. MacPhee, B. A. D. Stocker, *Clinical Res.* **17**, 157 (1969).
15. B. N. Ames and E. G. Gurney, unpublished; TA1532 has a deletion through the galactose operon and thus is sensitive to the bacteriophage C21. We have selected C21-resistant mutants of TA1532 and have chosen one (TA1537) which was as highly sensitive to crystal violet and deoxycholate as was an authentic deep rough strain. We are indebted to H. Nikaido for suggesting this procedure and for supplying the phage and deep rough strain.
16. B. N. Ames, E. G. Gurney, J. A. Miller, in preparation.
17. P. E. Hartman, K. Levine, Z. Hartman, H. Berger, *Science* **172**, 1058 (1971); B. N. Ames, unpublished data.
18. O. G. Fahmy and M. J. Fahmy, *Int. J. Cancer* **6**, 250 (1970).
19. D. L. Riddle and J. R. Roth, *J. Mol. Biol.* **54**, 131 (1970); *ibid.*, in press.
20. Supported by AEC grant AT(04-3)34 P.A.156 to B.N.A. and by grants to the Chester Beatty Research Institute (Institute of Cancer Research, Royal Cancer Hospital) from the Medical Research Council and the Cancer Research Campaign. We acknowledge the assistance of E. G. Gurney, Ben Tong, Frank Lee, and Anne Liggett.

10 December 1971

Radula Tooth Structure of the Gastropod Conus imperialis Elucidated by Scanning Electron Microscopy

Abstract. *Scanning electron microscopy of the hollow, harpoon-like radula tooth of the toxoglossan gastropod* Conus *has elucidated the structure and relationships of its component parts: apex, cutting edge, barbs, serration, adapical and basal openings of the lumen, external and internal folds of the shaft, and base. The functional roles of these components in prey capture are proposed.*

The radula of toxoglossan gastropods of the genus *Conus* is a simplified structure. It lacks the chitinous basal ribbon and associated musculature and supportive cartilage characteristic of this organ in most snails; it is quite short; and it has but two teeth in each row, only one of which is used at one time. However, these radula teeth are probably the most complex and highly modified in the Mollusca. Each tooth is adapted to perform at least three specialized roles almost simultaneously in capturing and overcoming prey: (i) the pointed apex must pierce the body wall of the prey; (ii) the tooth must catch and hold the body of the prey; and (iii) venom must be conveyed through the lumen of the tooth into the wound. In addition, in some species the base of the tooth must be held firmly by the tip of the proboscis during and after the injection process. The prey, typically polychaete annelids but other mollusks or fishes in some cases, is partially paralyzed by the venom and swallowed whole (*1*).

Although the general nature of the *Conus* radula tooth and something of the diversity of tooth form in the genus have been known for many years (*2, 3*), earlier studies have failed to elucidate the fundamental structural pattern of the tooth and the functional significance of its component features. This is due largely to the difficulty of determining the three-dimensional relationships of the internal and external parts of the enrolled, tubular, translucent, asymmetrical tooth by light microscopy (*2, 3*). By emphasizing surface details of teeth made opaque in preparation, and by af-