being estimated from its staining intensity on thin-layer chromatograms. These curves are coincident (Fig. 2), and the effects of the natural and synthetic peptides are therefore quantitatively indistinguishable.

We have bioassayed an additional set of tetra-, tri-, and dipeptides containing various permutations of the same three amino acids found in *Macrocallista* FMRFamide (*14*). Of these, only Phe-Met-Arg-Phe-OH and Met-Arg-Phe-$NH_2$ had any activity; the relative potencies were no better than, respectively, 1/100 and 1/1000 that of FMRFamide. The dependence of activity like that of peak C on the specific structure and sequence proposed by analysis of the natural product suggests, again, the identity of *Macrocallista* peak C and FMRFamide.

FMRFamide resembles a synthetic gastrin-(14-17)-tetrapeptide amide analog, Phe-Met-Asp-Phe-$NH_2$ (*15*). But, since FMRFamide contains an arginyl residue in place of the essential aspartyl residue of the gastrin analog, it should not have any gastrin-like activity. Immunoreactive gastrin has been found in the blood, and gastrointestinal tissue of two species of gastropod molluscs, but this substance differs from peak C both in its size and tissue distribution (*16*).

Peak C activity has been observed in all the major molluscan classes (*3*), but there is no evidence as yet that all peak C's are FMRFamide. Moreover, although FMRFamide is a highly active agent, and is highly localized in *Macrocallista* ganglia (*3*), its normal physiological role has still to be defined. About 20 years ago, Welsh (*17*) noted that, for many neurotransmitters, there is a polypeptide neurohormone that mimics its action. He suggested that these polypeptides might be serving as stable, long-range, and long-term substitutes for the fast-acting neurohumors. Given the similarity between the actions of 5-hydroxytryptamine and FMRFamide on molluscan heart, and assuming that FMRFamide is, in fact, a neurosecretory product, its physiological role in molluscs might be as a long-term regulator of muscular rhythmicity and tone.

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### References and Notes

1. N. Frontali, L. Williams, J. H. Welsh, *Comp. Biochem. Physiol.* **22**, 831 (1967).
2. Reviewed by M. J. Greenberg, R. A. Agarwal, L. A. Wilkens, P. J. B. Ligon, in *Neurobiology of the Invertebrates, Tihany 1971*, J. Salanki, Ed. (Akademiai Kiado, Budapest, 1973), p. 123.
3. R. A. Agarwal, P. J. B. Ligon, M. J. Greenberg, *Comp. Gen. Pharmacol.* **3**, 249 (1972).
4. D. A. Price, thesis, Florida State University (1977).
5. Phe, phenylalanine; Met, methionine; Arg, arginine; Asp, aspartic acid; the term gastrin-(14-17)-tetrapeptide indicates the sequence at residues 14 to 17.
6. The yield of a number of peak C preparations was estimated by various methods: ultraviolet absorbance, amino acid analysis, and staining intensity on thin-layer chromatograms. The results were similar; that is, 5 nmole per gram of dried ganglia, or 10 pmole per clam.
7. C. J. Bruton and B. S. Hartley, *J. Mol. Biol.* **52**, 165 (1970).
8. V. Neuhoff, *Micromethods in Molecular Biology* (Springer-Verlag, New York, 1973), p. 85.
9. "Trypsin exhibits the highest degree of substrate specificity known for an endopeptidase; only those bonds involving the carboxyl groups of lysyl and arginyl residues are hydrolyzed" [C. B. Kasper, in *Protein Sequence Determination*, S. B. Needleman, Ed. (Springer-Verlag, New York, ed. 2, 1975), p. 132].
10. K. R. Woods and K. T. Wang, *Biochim. Biophys. Acta* **133**, 369 (1967), as modified for the microscale by Neuhoff (*8*).
11. F, M, and R are the one-letter abbreviations for the amino acids phenylalanine, methionine, and arginine approved by the IUPAC-IUB Commission on Biochemical Nomenclature [*Pure Appl. Chem.* **31**, 641 (1972)]. Thus, the designation FMRFamide conveys both the amino acid sequence of the peptide and the fact that the carboxyl terminal is substituted with a amide group.
12. Obtained from Research Plus Laboratories, Denville, N.J.
13. Eastman No. 6064 cellulose chromatogram sheets were used with a solvent consisting of *n*-butanol, acetic acid, and water (4 : 1 : 2), by volume. Spots were visualized with ninhydrin and the Sakaguchi reagent.
14. The following peptides were purchased: Met-Arg-Phe-OH (*12*), Arg-Phe-OH (*12*), and Phe-Arg-OH (Cyclo Chemical, Los Angeles, Calif.). Phe-Met-Arg-Phe-OH and its amide, Met-Arg-Phe-$NH_2$, and Arg-Phe-$NH_2$ were synthesized from these purchased peptides. Phe-Met-Phe-Arg-OH and Phe-Met-Arg-OH and its amide were synthesized from the component-protected amino acids.
15. J. S. Morley, *Proc. Roy. Soc. London Ser. B* **170**, 97 (1968).
16. E. Straus, R. S. Yalow, H. Gainer, *Science* **190**, 687 (1975).
17. J. H. Welsh, in *The Hormones* G. Pincus and K. V. Thimann, Eds. (Academic Press, New York, 1955), vol. 3, p. 281.
18. Supported by NIH grant HI09283 to M.J.G. This is contribution 70 from the Tallahassee, Sopchoppy, and Gulf Coast Marine Biological Association.

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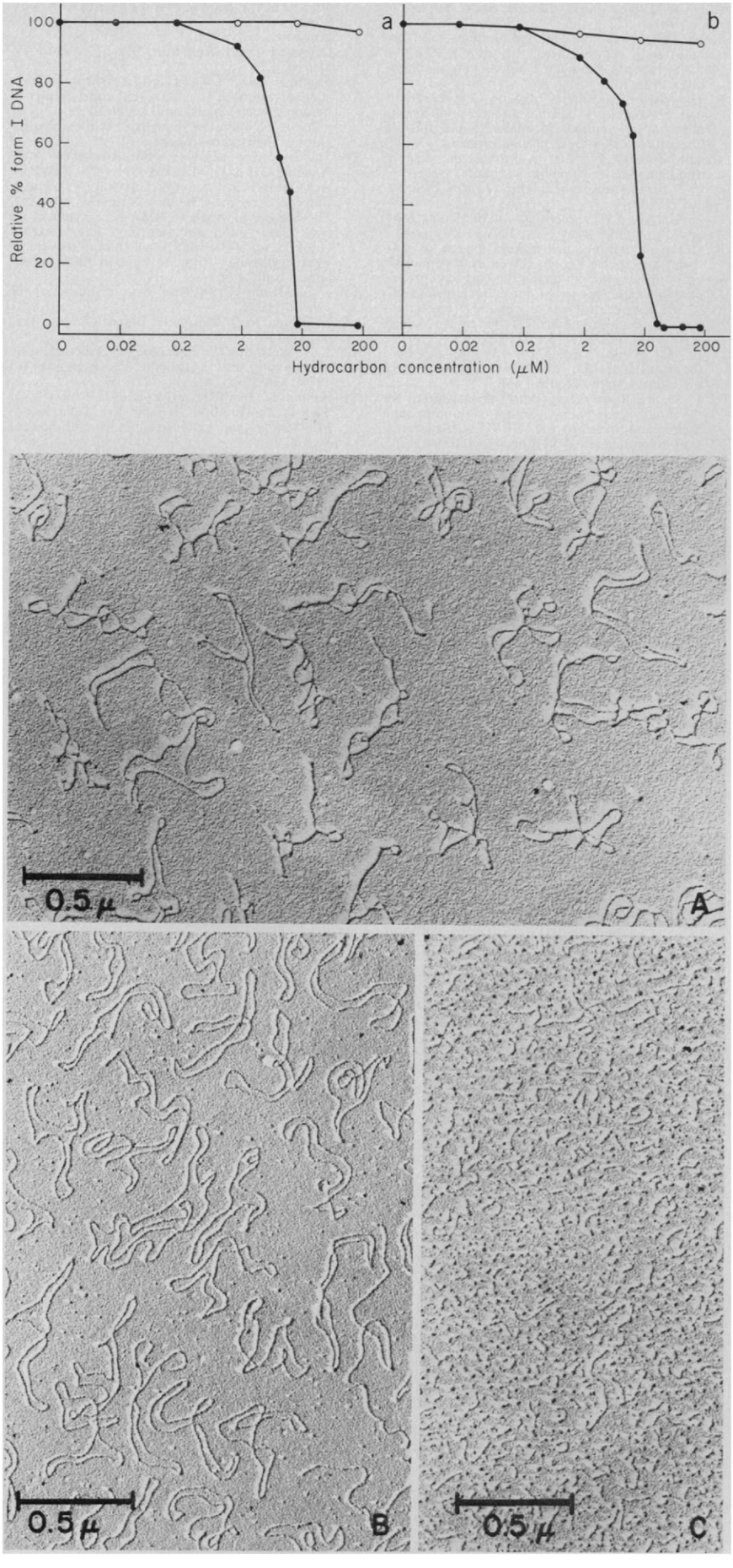
## DNA Strand Scission by Benzo[*a*]pyrene Diol Epoxides

Abstract. Syn-*and* anti-*benzo*[a]*pyrene diol epoxides elicit a concentration-dependent nicking of superhelical Col E1 DNA in an in vitro reaction monitored by agarose gel electrophoresis and electron microscopy. This strand scission represents less than 1 percent of the DNA modification by diol epoxide. Kinetic analysis implicates the formation of unstable phosphotriesters, hydrolysis of which nick the DNA.*

Benzo[*a*]pyrene is a widespread environmental pollutant possessing potent mutagenic and carcinogenic activity. This activity is dependent on metabolic activation of the hydrocarbon by microsomal monooxygenase. Recent evidence suggests that a 9,10-oxide of *trans*-7,8-dihydro-7,8-dihydroxybenzo[*a*]pyrene may be the ultimate carcinogenic metabolite (*1*). It appears that microsomal activation proceeds stereospecifically to give the *anti*-diol epoxide (*2, 3*). Both diastereomers, however, react with nucleic acids and are highly mutagenic (*3, 4*). Weinstein *et al.* (*5*), working with the *anti* isomer, and Koreeda *et al.* (*6*), working with the *syn* isomer, characterized the primary RNA adduct as a linkage between the C-2 amino group of guanine and the C-10 position of the hydrocarbon. Reaction of diol epoxide with DNA has not been characterized. We report here that both diastereomeric diol epoxides degrade DNA and RNA and present a mechanism for how this process might occur.

Covalently closed superhelical DNA, such as the *Escherichia coli* plasmid Col E1, is a sensitive probe for detecting strand scission. Form I Col E1 DNA, with a molecular weight of $4.2 \times 10^6$, contains more than 12,000 phosphodiester linkages (*7*); cleavage of any one permits the DNA strands to unwind, resulting in relaxed form II DNA. Introduction of another nick adjacent to the first but on the opposite strand gives linear form III DNA. All three forms are conveniently resolved by agarose gel electrophoresis. The Col E1 DNA used in this study contains 0.3 percent RNA as a discrete segment (*7*). Similar nicking, however, is seen with RNA-free SV40 DNA.

When Col E1 DNA is reacted with either diastereomeric diol epoxide in tris-HCl buffer, *p*H 8.0, gel electrophoresis shows substantial nicking (Fig. 1). A minimum ratio of diol epoxide to DNA mononucleotide of 0.01 is required for detectable nicking. Above this value nicking increases, until at a ratio of 0.5 to 1.0 no form I DNA remains. The effects of strand scission are also observed by electron microscopy (Fig. 2). When form I DNA is reacted with the *anti* isomer, relaxed circles result from a ratio of 0.5, while linear segments of random length result from a ratio of 5. At even higher ratios of diol epoxide to mononucleotide (that is, 15) a significant fraction of the DNA (~ 20 percent) becomes acid soluble, presumably due to the release of small oligonucleotides. This increased fragmentation suggests that nicks are occurring at many sites around the molecule and are not restricted to the RNA segment mentioned above. When the reaction is carried out in 20 m*M* phosphate




buffer, *p*H 7.3, nicking is detectable only at ratios of diol epoxide to mononucleotide greater than 1.3. This probably reflects direct reaction of diol epoxide with inorganic phosphate (*6*, *8*). Hydrolysis of the diol epoxides results in tetraols, which lack nicking activity (Fig. 1).

By scaling up the reaction mixtures 50-fold and employing $^{14}C$-labeled *anti*-diol epoxide it was possible to estimate the relative frequency of strand scission compared to adduct formation in Col E1 DNA. Nicking was quantified by agarose gel electrophoresis of the DNA, whereas covalently bound hydrocarbon was determined by counting the DNA pellet after repeated ethyl acetate and hot ethanol extractions. Multiple determinations at several ratios of diol epoxide to DNA mononucleotide were made. Nicking is an infrequent event, with 330 ± 30 adducts formed per strand scission. At a ratio of diol epoxide to DNA mononucleotide of 0.5 the resultant form II Col E1 DNA has 310 ± 20 adducts per molecule. At a ratio of 0.05, where less than 15 percent of the DNA is nicked, there are 47 ± 4 adducts per molecule.

RNA is also degraded by the diol epoxide. For example, when MS2 RNA (~ $1.0 \times 10^6$ daltons) is reacted with *anti*-diol epoxide at a ratio of hydrocarbon to mononucleotide of 5.0 and then analyzed by agarose gel electrophoresis, it migrates rapidly as a diffuse low-molecular-weight band. Since alkylated bases in RNA are not readily lost to give apurinic or apyrimidinic sites, RNA strand scission is considered diagnostic for the presence of phosphotriesters (*9*).

Fig. 1 (top left). Nicking of superhelical Col E1 DNA by benzo[*a*]pyrene diol epoxides. Superhelical Col El DNA (mononucleotide concentration, 32.5 μ*M*) in 20 m*M* tris-HCl, *p*H 8.0, containing 0.5 m*M* EDTA and 5 percent (by volume) dimethyl sulfoxide was incubated at 37°C with the indicated concentrations of diol epoxide (closed circles) or tetraol (open circles). After 24 hours, 40-μl portions were placed on a 1.4 percent agarose slab gel and subjected to electrophoresis at 50 volts for 18 hours. The gel was stained with ethidium bromide, and the DNA bands were quantified with a Schoeffel model SD3000 spectrodensitometer in the reflectance mode. (a) The *anti*-diol epoxide and its tetraol hydrolysis product; (b) the *syn*-diol epoxide and its tetraol hydrolysis product. *Syn*- and *anti*-diol epoxides were synthesized as described (*4*, *19*). Fig. 2 (bottom left). Electron micrographs of Col E1 DNA (mononucleotide concentration, 32.5 μ*M*) reacted with (A) 0, (B) 16.5, and (C) 165 μ*M* *anti*-diol epoxide. The DNA was visualized by the Kleinschmidt technique with the use of a unidirectional Pt/Pd shadow. With this technique, hydrocarbon aggregates appear as small background granules.

The hydrolysis of such triesters is catalyzed by the 2′-hydroxyl group of the sugar. Phosphotriester half-life depends on the nature of the adduct but may be as long as several hours (*10*). RNA fragmentation by diol epoxide is thus indicative of phosphotriester formation. Indirect evidence presented by Koreeda *et al.* (*6*) for reaction of *syn*-diol epoxide with the phosphate groups of polyguanylate [poly(G)] supports this conclusion.

In DNA, fragmentation is normally attributed to depurination and depyrimidination strand scission (*9, 11*). Electrophilic attack at guanine N-7, adenine N-3, or pyrimidine O-2 introduces a formal positive charge into the ring. This labilizes the glycosidic linkage and leads to loss of the modified bases. The resultant apurinic or apyrimidinic sites can slowly rearrange to give strand scission. In DNA, phosphotriesters are stable species resulting from the absence of 2′-hydroxyl groups (*12*). However, the presence of a β-hydroxyl group on the electrophile could catalyze triester hydrolysis and concomitant strand scission (*13*). By analogy with RNA, diol epoxide probably forms phosphotriesters in DNA. If so, the C-9 hydroxyl group of the hydrocarbon should facilitate triester hydrolysis with strand scission. DNA degradation by diol epoxide could thus proceed by two different mechanisms.

The kinetics of DNA nicking by diol epoxide supports the phosphotriester mechanism. In aqueous solution at *p*H 8.0 and 37°C, *anti*-diol epoxide has a half-life of 21 minutes, which effectively limits its reaction with DNA to a 2-hour period (*14*). Strand scission is a secondary process contingent on initial adduct formation. When Col E1 DNA is reacted with *anti*-diol epoxide at a ratio of hydrocarbon to mononucleotide of 0.5, each DNA molecule is nicked an average of once and the entire relaxation process is complete in 4 hours (Fig. 3). The rapidity with which nicking follows adduct formation is not typical of depurination or depyrimidination strand scission. These processes proceed slowly, as exemplified by the nicking of Col E1 DNA by dimethyl sulfate (Fig. 3). This alkylating agent, with a half-life of 10 minutes in aqueous solution, reacts primarily with guanine N-7 (*15*). While it is conceivable that the *N*-glycosidic linkage to a specific hydrocarbon-base adduct could be rapidly cleaved to give an apurinic or apyrimidinic site, subsequent rearrangement of the sugar to give strand scission is known to be a slow process under physiological conditions (*16*). Acceleration of the rate of rearrangement by the presence of diol epoxide or tetraol is unlikely. We therefore suggest that facile phosphotriester hydrolysis is the most likely explanation for the rapid nicking of DNA by diol epoxide.

A mechanism for phosphotriester hydrolysis is presented in Fig. 4. Since DNA phosphate does not readily displace electrophiles, ester formation at the C-10 position of the hydrocarbon most likely proceeds through an SN1 mechanism (*17*). The C-9 hydroxyl group can then displace one of the sugars, thereby breaking the DNA backbone and forming a cyclic triester. Tertiary cyclic phosphates, like the one proposed, hydrolyze rapidly to relieve ring strain (*18*). Upon hydrolysis the hydrocarbon remains attached to the phosphate. Since the β-hydroxyl group is on the hydrocarbon and not the sugar, each triester hydrolysis gives a nick.

If confirmed, the formation of unstable deoxyribophosphotriesters by benzo[*a*]pyrene diol epoxide is a unique reaction. The low frequency of phosphotriester formation in vitro does not preclude the existence of triesters in vivo, where they may play a role in the high mutagenic and potentially carcinogenic activity of diol epoxide.

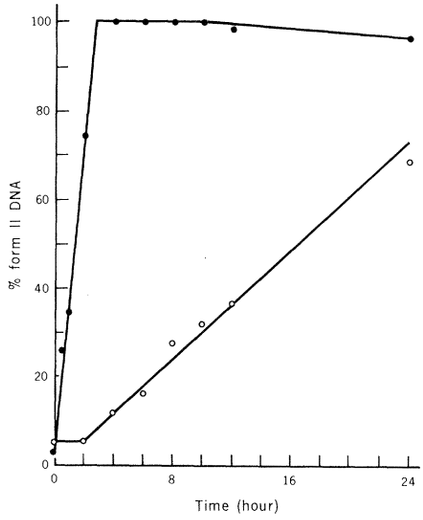



Fig. 3. Kinetics of Col E1 DNA relaxation by the *anti*-diol epoxide and dimethyl sulfate. Superhelical Col E1 DNA (mononucleotide concentration, 32.5 μ*M*) was reacted with 16.5 μ*M* *anti*-diol epoxide (closed circles) or 530 μ*M* dimethyl sulfate (open circles) in 20 m*M* tris-HCl, *p*H 8.0, containing 0.5 m*M* EDTA and 5 percent (by volume) dimethyl sulfoxide at 37°C. Samples were taken at various times for analysis by agarose gel electrophoresis.

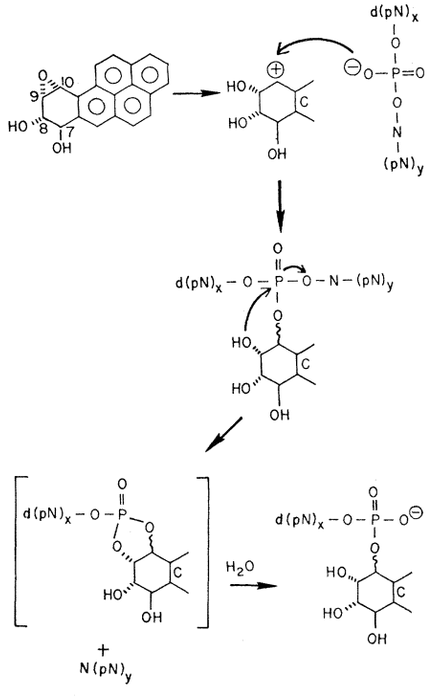



Fig. 4. Postulated mechanism of DNA strand scission by benzo[*a*]pyrene diol epoxides.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### References and Notes


1. A. Borgen, H. Darvey, N. Castagnoli, T. T. Crocker, R. E. Rasmussen, I. Y. Wang, *J. Med. Chem.* **16**, 502 (1973); P. Sims, P. L. Grover, A. Swaisland, K. Pal, A. Hewer, *Nature (London)* **252**, 326 (1974); P. Daudel, M. Duquesne, P. Vigny, P. L. Grover, P. Sims, *FEBS Lett.* **57**, 250 (1975); V. Ivanovic, N. E. Geacintov, I. B. Weinstein, *Biochem. Biophys. Res. Commun.* **70**, 1172 (1976); T. Meehan, K. Straub, M. Calvin, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1437 (1976).
2. *Syn*- and *anti*-diol epoxide refer, respectively, to (±)-7α,8β-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene and (±)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.
3. E. Huberman, L. Sachs, S. K. Yang, H. V. Gelboin, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 607 (19 → S. K. Yang *et al.*, *ibid.*, p. 2594.
4. C. Malaveille, H. Bartsch, P. L. Grover, P. Sims, *Biochem. Biophys. Res. Commun.* **66**, 693 (1975); P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin, H. Yagi, O. Hernandez, D. M. Jerina, A. H. Conney, *ibid.* **68**, 1006 (1976); → H. Yagi, O. Hernandez, D. M. Jerina, *J. Am. Chem. Soc.* **97**, 6881 (1975).
5. I. B. Weinstein, A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, R. G. Harvey, C. Harris, H. Autrup, H. Kasai, K. Nakanishi, *Science* **193**, 592 (1976).
6. M. Koreeda, P. D. Moore, H. Yagi, H. J. C. Yeh, D. M. Jerina, *J. Am. Chem. Soc.* **98**, 6720 (1976).
7. D. B. Clewell and D. R. Helinski, *Biochemistry* **9**, 4428 (1970); P. H. Williams, H. W. Boyer, D. R. Helinski, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3744 (1973).
8. D. R. Thakker, H. Yagi, A. Y. H. Lu, W. Levin, A. H. Conney, D. M. Jerina, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3381 (1976).
9. K. V. Shooter, *Chem. Biol. Interact.* **11**, 575 (1975); B. Singer, *Nature (London)* **264**, 333 (1976).
10. B. Singer and H. Fraenkel-Conrat, *Biochemistry* **14**, 772 (1975).
11. B. Singer, *Prog. Nucleic Acid Res. Mol. Biol.* **15**, 219 (1975).
12. P. Bannon and W. Verly, *Eur. J. Biochem.* **31**, 103 (1972).
13. S. Walles and L. Ehrenberg, *Acta Chem. Scand.* **22**, 2727 (1968).
14. The hydrolysis of *anti*-diol epoxide was fol-

lowed by an indirect assay based on the ability of diol epoxide to nick supercoiled Col E1 DNA. The half-life reported here is substantially longer than values obtained by Wood *et al.* who used mutagenesis and cytotoxicity assays [A. W. Wood, P. G. Wislocki, R. L. Chang, W. Levin, A. Y. H. Lu, H. Yagi, O. Hernandez, D. M. Jerina, A. H. Conney, *Cancer Res.* **36**, 3358 (1976)].
15. K. V. Shooter, *Chem. Biol. Interact.* **13**, 151 (1976); ——— and R. K. Merrifield, *ibid.*, p. 223.
16. B. Strauss, M. Coyle, M. Robbins, *Cold Spring Harbor Symp. Quant. Biol.* **33**, 277 (1968); H.-J. Rhaese and E. Freese, *Biochim. Biophys. Acta* **190**, 418 (1969).
17. K. V. Shooter, R. Howse, S. A. Shah, P. D. Lawley, *Biochem. J.* **137**, 303 (1974); K. V. Shooter, R. Howse, R. K. Merrifield, *ibid.*, p. 313.
18. D. M. Brown, D. I. Magrath, A. R. Todd, *J. Chem. Soc.* (1955), p. 4396.
19. D. J. McCaustland and J. F. Engel, *Tetrahedron Lett.* (1975), p. 2549.
20. Col E1 DNA was obtained from Professor J. Hearst of the University of California at Berkeley. $^{14}$C-Labeled *anti*-diol epoxide (53.7 mc/mmole) was obtained from Midwest Research Institute. We thank Dr. T. Meehan for critical reading of the manuscript. Supported by National Cancer Institute contract YOI-CP50203 and the Division of Biomedical and Environmental Research of the U.S. Energy Research and Development Administration.

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## Interferon: An Inducer of Macrophage Activation by Polyanions

Abstract. *Purified mouse fibroblast interferon (IF) directly rendered resting macrophages tumoricidal. The physicochemical properties and species specificity of the stimulatory agent fall within the present definition of IF. Since a number of polyanions induce macrophage IF, the antitumor and antimicrobial activities may result from the ability of newly released IF to modify macrophage activity.*

Macrophages appear to have an extremely important if not crucial role in maintaining the purity of the internal environment, in surveillance against spontaneously arising malignant cells (*1*), and in controlling tumor cell growth and dissemination [for review, see (*2*)]. A number of agents allow the macrophage to reach an "activated" state whereby the cell exhibits increased phagocytosis (*3*), elevated degradative enzyme activities (*4*), and enhanced cytostatic and cytocidal effects on transformed cells (*5–7*). We have previously shown that nonspecific macrophage activation correlates well with the capacity to enhance host resistance against neoplasia (*6*). Macrophages recovered from these treated animals were selectively cytotoxic for tumor cells by a nonphagocytic contact mechanism which is poorly understood.

In view of these findings on macrophage activity following drug administration in vivo, it seemed appropriate to study further whether these agents might directly activate macrophages in vitro. This information would greatly aid in understanding the mechanism of macrophage activation and may allow for the identification of pharmacologic agents which possess the ability to specifically modify the functional activity of the reticuloendothelial system.

We used a modification of the method of Alexander and Evans (*8*) to measure the ability of drugs to directly activate macrophages in vitro (*9*). Male $CD_2F1$ mice were obtained from the Mammalian Genetics and Animal Production Section of the National Institutes of Health, Bethesda, Maryland. Noninduced peritoneal exudates were harvested with heparinized medium, and macrophages were purified by adherence. Approximately $1 \times 10^6$ macrophages were seeded in 3.5-cm Falcon plastic dishes in 1 ml of standard growth medium consisting of RPMI-1640 supplemented with 20 percent fetal calf serum. An established line of MBL-2 leukemia cells was adjusted to $5 \times 10^4$ cells per milliliter of growth medium, and 2-ml portions were immediately admixed with the macrophage cultures. Drugs were made up in tenfold concentrations, and 0.3 ml was added to the cell mixtures. All cultures were incubated at 37°C in an atmosphere of 5 percent $CO_2$ in air, and viable leukemia cells were counted daily with a hemacytometer. The ratio of macrophages to target cells was 10:1 at the beginning of each experiment. The percentage of growth inhibition of MBL-2 cells due to macrophage-drug interaction was calculated by comparison to MBL-2 cells grown in the presence of normal macrophages alone.

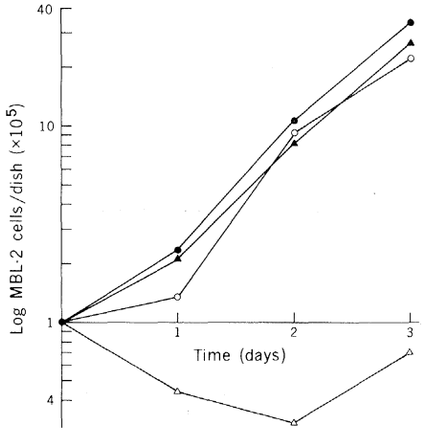


Fig. 1. Effect of peritoneal macrophages and purified mouse interferon on the growth of MBL-2 leukemia cells in vitro. MBL-2 cells ($10^5$) were grown in 3 ml of RPMI-1640 growth medium. (•) Leukemia cells alone; (○) leukemia cells in the presence of $10^6$ normal macrophages; (▲) leukemia cells in the presence of IF (1000 units per milliliter of growth medium); (△) leukemia cells plus $10^6$ macrophages plus 1000 units of IF per milliliter of growth medium.

Using the system described above, we showed that pyran copolymer, poly(I) · poly(C) (polyinosinylate · polycytidylate) and dextran sulfate rendered macrophages cytotoxic for leukemia cells in vitro (*9*). The effect was dose-dependent and required greater than 24 hours after exposure to drug. Similarly, the administration in vivo of these agents produces a highly dose-dependent macrophage activation (*10*). The growth inhibition of tumor cells appeared to result from a modification of the macrophages themselves, since neither macrophages nor drug alone interfered with MBL-2 proliferation. The primary mechanism of cytotoxicity did not involve toxic drug metabolites or cytotoxins liberated by the macrophage cultures (*9*). Cell-to-cell contact appeared to be involved in cytostasis since tumor cells were observed to aggregate around and adhere firmly to drug-activated macrophages.

The mechanism of direct activation of macrophages by these nonspecific agents appeared to be related to the common polyanionic character of these molecules. We therefore examined a variety of polyanions for the ability to render macrophages cytotoxic (Table 1). Whereas heparin and poly(A) · poly(U) (polyadenylate · polyuridylate) had a similar stimulatory effect ($P < .001$) on macrophage function, single-stranded RNA was not effective. Control agents consisting of DEAE-dextran, levamisole, and bovine serum albumin were without significant effect.

In view of these observations and the fact that macrophages elaborate and secrete interferon (IF) after polyanion treatment (*11*), we examined the question of whether IF was involved in macrophage activation. Recent observations that crude IF preparations can enhance macrophage phagocytosis in vivo (*12*) and in vitro (*13*) and that the lymphokines (migration inhibition factor and type II IF) cannot be physically separated (*14*) support the concept that IF may regulate macrophage activity. Of particular interest are the findings of Gresser *et al.* that (i) the antitumor effects of IF are host-mediated and not due to direct inhibitory effects on tumor cell growth (*15*) and (ii) that phagocytosis of tumor cells by macrophages was ob-