*THE INFLUENCE OF POLYCYCLIC AROMATIC HYDROCARBONS ON BACTERIOPHAGE DEVELOPMENT, II**

By Wen-Tah Hsu, John W. Moohr, Albert Y. M. Tsai, and Samuel B. Weiss

ARGONNE CANCER RESEARCH HOSPITAL† AND DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CHICAGO

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A recent publication from this laboratory[1] described the inhibitory effect of certain aromatics on the replication of single-stranded RNA[2] and DNA bacterial viruses using the isolated nucleic acid as the infectious agent. At the same time, it was observed that most of the aromatics that exhibited this effect were also carcinogenic. The viral replication response to hydrocarbons requires the use of infected bacterial spheroplasts; no effect is observed with intact infected bacteria. Although the mechanism by which the aromatics exert their inhibitory influence is unclear, it appears that the active hydrocarbons do not inhibit the infectious process itself or the release of newly formed viruses, nor do they induce new enzymes inhibitory for viral production.

The present communication provides further information on the nature of the hydrocarbon response in infected and noninfected *Escherichia coli* spheroplasts. A method is described for preparing infected spheroplasts from cells preinfected with phage which permits one to assay the hydrocarbon effect on the replication of both single- and double-stranded viruses whereby significant differences are observed. The present study demonstrates the inhibition of viral nucleic acid and protein synthesis by 7,12-DMBA, and, in contrast to one of our earlier observations, we now report that 7,12-DMBA inhibits nucleic acid and protein synthesis in the bacterial host itself. The protective effect of various aromatics on 7,12-DMBA-induced viral inhibition is also reported.

Methods.—Bacterial viruses and infectious RNA: MS2 and lambda (λ) phage were grown on the male strain of *E. coli* K12W1485; ϕX174 and T4 phage were grown on *E. coli* C and *E. coli* B, respectively. Infectious MS2 RNA was isolated from MS2 phage by phenol extraction[3] and stored in 5 mM EDTA, pH 7.4, at $-20°$.

Preparation of infected spheroplasts with chloramphenicol (CA method): The growth of *E. coli* cells was previously described.[1] To 35 ml of culture at 5×10^8 cells/ml in 3XD medium,[4] 0.7 ml of chloramphenicol (1 mg/ml) and 0.05 ml of 1 *M* $CaCl_2$ were added, and the mixture was allowed to stand for 5 min. Bacteriophage was then added at a ratio of 10 PFU per bacterium for MS2, λ, and ϕX174, and 4 PFU per bacterium for T4, and adsorption was allowed to proceed for another 5 min. The mixture was then chilled in ice and centrifuged at 4°. The packed cells were washed once with 10 ml of 3XD medium containing chloramphenicol (20 μg/ml), and then suspended in a mixture containing 0.1 ml of chloramphenicol (10 μg), 0.7 ml of 50% sucrose, 0.02 ml of lysozyme (40 μg), and 0.4 ml of 5 mM EDTA. This mixture was incubated at 30° for 6 min, after which 20 ml of cold PAM medium,[5] modified to contain no peptone, was added. The cells were sedimented in the cold by centrifugation and then resuspended in 20 ml of the modified cold PAM medium without chloramphenicol. These infected spheroplasts were used directly for testing the hydrocarbon response (see below).

Preparation of noninfected spheroplasts: E. coli K12W1485 were converted to spheroplasts with lysozyme and EDTA as previously described,[1] except that 0.20 ml of 0.25 *M* Tris, pH 8.1, was substituted for bovine serum albumin during the lysozyme-EDTA treatment and the spheroplasts were used almost immediately after preparation, instead of standing for 1 hr at room temperature.

Hydrocarbon additions to spheroplasts and viral assay: Stock solutions of hydrocarbon were

prepared in dimethylformamide. Appropriate dilutions of the aromatic solutions were made in PAM medium for addition to spheroplasts; the dimethylformamide concentration did not exceed 0.4% in the final assay system. Control assays (no hydrocarbon) contained the same amount of solvent. For the "CA method" of infection, the hydrocarbon suspension was added to an appropriate quantity of infected spheroplasts and then incubated at 30°. After certain times of incubation, cells were removed and lysed by shaking with 3XD medium containing $CHCl_3$ and 0.01 *M* NaCN. They were then assayed for phage titer by the agar layer technique.[6] The indicator bacteria used for the different phages were the strains of *E. coli* on which the viruses had been grown. For all assays, the tubes containing the infectious mixtures were wrapped in aluminum foil to exclude light.

Studies with radioactive substrates: For studies on C^{14}-uracil and C^{14}-amino acid incorporation into nucleic acid and protein, the labeled compounds were added to spheroplasts, and the cells were then treated at appropriate times with 5% trichloroacetic acid. For RNA determinations, the cold-precipitable material was collected by centrifugation, washed several times with cold acid, and then collected on Millipore filters, dried, and counted. For protein determinations, the acid-precipitable material was treated in a similar manner, except that it was also subjected to heating at 90° for 20 min in 5% acid before isotope determination.

Viral RNA polymerase: Cells of the lysis-inhibited male strain of *E. coli* C3000 were infected with MS2 phage and converted to spheroplasts by the "CA method" described above. The cells were diluted twofold with PAM medium, and, after gentle shaking for 60 min at 30° in the presence or absence of hydrocarbon, collected by centrifugation, rinsed once with 10% sucrose, lysed in a hypotonic medium containing 0.01 *M* Tris (pH 8.1), 0.01 *M* mercaptoethanol, and 0.001 *M* EDTA and centrifuged at 30,000 × *g*. The supernatant collected after centrifugation was assayed for its ability to catalyze the RNA-dependent synthesis of RNA as described by Lodish, Cooper, and Zinder.[7]

Radioactive uracil-2-C^{14} was purchased from Schwarz BioResearch, Inc.; C^{14}-amino acids were obtained from New England Nuclear Corp.; actinomycin D was a gift from Merck and Co., Inc.

Results.—The effect of aromatics on single-stranded RNA and DNA phage replication: Table 1 shows the effect of various polycyclic aromatic hydrocarbons on the replication of MS2 and φX174 when infected spheroplasts are prepared by the "CA method." It may be seen that certain aromatics strongly inhibit virus production, while others have little or no effect. Except for the aromatic 1,12-DMBA, which is noncarcinogenic in rat, those hydrocarbons that strongly suppress viral replication also exhibit biological activity in higher organisms. The reverse correlation is equally impressive; hydrocarbons that are inactive as viral inhibitors are also in-

TABLE 1

EFFECT OF VARIOUS HYDROCARBONS ON MS2 AND φX174 PHAGE PRODUCTION

Hydrocarbon	MS2 Phage: Phage titer (PFU/ml × 10^{-7})	MS2 Phage: Per cent inhibition	φX174 Phage: Phage titer (PFU/ml × 10^{-7})	φX174 Phage: Per cent inhibition	Biological* activity in rats
None	410	0	89	0	−
BA	380	7	84	6	−
7,12-DMBA	39	91	22	75	+
6,8-DMBA	38	91	17	81	+
1,12-DMBA	12	97	18	80	−
3,9-DMBA	410	0	88	1	−
4-MBA	390	5	89	0	−
5-MBA	330	19	73	18	−
6-MBA	24	94	12	86	+
Chrysene	410	0	78	12	−

Infectious spheroplasts were prepared by the "CA method." The hydrocarbon assay mixture contained 0.1 ml of the infected spheroplast preparation (see *Methods*) and 0.9 ml of PAM medium containing 20 μg of the appropriate hydrocarbon as indicated above. After incubation at 30° for 3 and 2 hr for the MS2 and φX174 infectious mixtures, respectively, the cells were lysed and titrated for whole phage as described under *Methods*.

* +, Produces sarcoma when 2.5 mg of hydrocarbon in 0.5 ml of sesame oil is injected in muscle of rat.

active as carcinogenic inducers. These results are similar to those reported previously, when the isolated viral nucleic acids instead of the intact bacteriophage were used as the infecting agents.[1]

Single-burst experiments were performed with MS2-infected cells to determine what proportion of the cells still produce virus in the presence of 7,12-DMBA, and whether the efficiency of virus production is altered. These experiments indicate that in the presence of 7,12-DMBA less than half of the infected cells give rise to phage, and the viral burst size per infected bacteria which do produce virus is also lower by a factor of more than two. The over-all hydrocarbon inhibition on plaque yield, therefore, results from a combination of these two effects.

Protection against the 7,12-DMBA response: When *E. coli* spheroplasts are pretreated with certain aromatics prior to exposure to 7,12-DMBA, the viral inhibition response is partially masked (Table 2). The aromatics that partially block the 7,12-DMBA inhibition appear to be those that are not inhibitors themselves and they are not carcinogenic in rat. Remarkably, in one instance when benz(a)anthracene was added after 7,12-DMBA preincubation, protection against viral inhibition was also observed. These results suggest some type of reversible competition between the aromatics for the active sites responsible for bacteriophage replication. The protection phenomenon by various aromatics on 7,12-DMBA-induced viral inhibition is extremely interesting, since a similar effect has been reported for DMBA-induced cancers in rats.[8, 9]

Viral nucleic acid synthesis: MS2 RNA synthesis was observed by determining the rate of C^{14}-uracil incorporation into acid-insoluble material in the presence of actinomycin D (Fig. 1). The antibiotic was included in the infected spheroplast mixture to suppress the DNA-directed RNA synthesis of the host; its effectiveness is demonstrated by the low level of uracil incorporation obtained with noninfected cells. On the other hand, MS2-infected cells show an active incorporation of labeled uracil into the RNA fraction in the presence of actinomycin D, suggesting that the rate of this incorporation reflects the rate of viral RNA synthesis. Under the same conditions, 7,12-DMBA lowers the rate of uracil incorporation. Assays for the appearance of infectious RNA throughout the period of viral development also indicate that the rate and extent of viral RNA synthesis are similarly suppressed by

TABLE 2

PROTECTION AGAINST 7,12-DMBA INHIBITION OF MS2 REPLICATION BY VARIOUS AROMATICS

Aromatic Treatment of Spheroplasts Prior to Infection with MS2 RNA		
First 30 min	Second 60 min	PFU/ml
None	None	7690
None	7,12-DMBA (25 μg/ml)	149
7,12-DMBA (2.5 μg/ml)	7,12-DMBA (25 μg/ml)	111
BA (25.0 μg/ml)	7,12-DMBA (25 μg/ml)	3590
3,9-DMBA (25.0 μg/ml)	7,12-DMBA (25 μg/ml)	4785
6,8-DMBA (25.0 μg/ml)	7,12-DMBA (25 μg/ml)	280
Chrysene (25.0 μg/ml)	7,12-DMBA (25 μg/ml)	2875
6-Aminochrysene (25.0 μg/ml)	7,12-DMBA (25 μg/ml)	257
7,12-DMBA (25.0 μg/ml)	BA (25 μg/ml)	3000

E. coli spheroplasts were incubated with various hydrocarbons (concentrations shown above) at 30° prior to infection. The hydrocarbons incubated with the cells for the first 30 min are shown in the first column. 7,12-DMBA or BA was then added to the mixtures and incubation continued for another 60 min. MS2 RNA (50 mμg in 0.50 ml) was then added to 0.50 ml of the hydrocarbon-treated spheroplasts, and the infectious mixtures were assayed for PFU after 20 min incubation at 30°. Each figure represents the average of duplicate assays.

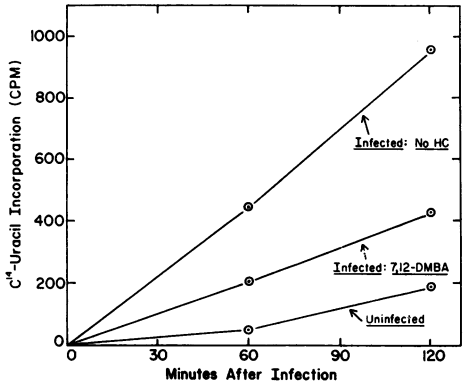

FIG. 1.—The effect of 7,12-DMBA on C^{14}-uracil incorporation by MS2-infected cells. The reaction mixture (4.84 ml) in the modified PAM medium contained *E. coli* spheroplasts (approximately 4×10^8 cells, either infected or noninfected); 97 μg of 7,12-DMBA; 0.1 μmole of uracil-2-C^{14} (45 mc per μmole); and 100 μg of actinomycin D. The mixtures were incubated at 30° and 0.60-ml samples were withdrawn at various times and the C^{14} content in the acid-precipitable fraction was determined as described under *Methods*. Phage titers on separate samples showed a 7,12-DMBA inhibition of 61% at 240 min after infection.

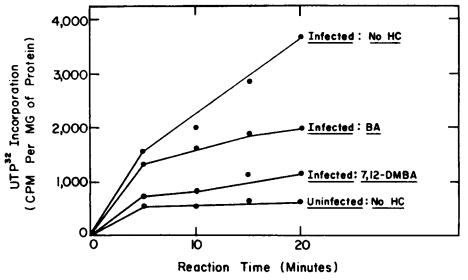

FIG. 2.—The effects of aromatics on MS2 RNA polymerase activity. The preparation of noninfected and MS2-infected spheroplast extracts, treated or nontreated with hydrocarbons, is described under *Methods*. The reaction mixture (2.6 ml) contained 300 μmoles of phosphate buffer (pH 7.5); 24 μmoles of $MgCl_2$; 60 μmoles of mercaptoethanol; 3.3 μmoles each of ATP, CTP, and GTP; 2.9 μmoles of UTP-α-P^{32} (13×10^6 cpm per μmole); 12 μmoles of phosphoenol pyruvate; 24 μg of pyruvate kinase; 12 μg of DNase; 30 μg of actinomycin D; and 0.60 ml of a 30,000 $\times$ *g* spheroplast extract. Aliquots were removed at various times, and the label incorporated into the acid-precipitable fraction was determined. The incorporation data were adjusted to correct for the slight differences in protein content of each sample assayed.

7,12-DMBA. Because of the reduced RNA synthesis observed, assays for viral RNA polymerase activity (induced by virus infection) were performed on extracts of infected cells. Figure 2 shows the kinetics of UTP^{32} incorporation into RNA catalyzed by a 30,000 $\times$ *g* extract prepared from MS2-infected spheroplasts in the presence of actinomycin D and pancreatic DNase. The low level of UTP^{32} incorporation observed with extracts from noninfected cells indicates the effectiveness of the antibiotic and DNase in blocking *E. coli* RNA polymerase activity (*lower curve*). Extracts prepared from MS2-infected cells, exposed to benz(a)anthracene or 7,12-DMBA, catalyzed less label incorporation than extracts from cells that had not been exposed to the aromatics; 7,12-DMBA is particularly effective in this respect.

The effect of hydrocarbons on viral protein synthesis: Figure 3 illustrates the effect of 7,12-DMBA on the incorporation of a mixture of labeled amino acids into the protein fraction of MS2-infected spheroplasts. It is apparent that the rate and extent of labeled amino acids incorporated is significantly lowered by the hydrocar-

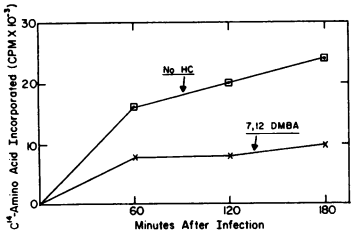

FIG. 3.—The effect of 7,12-DMBA on the incorporation of C^{14}-amino acids by MS2-infected cells. The reaction mixture (1 ml) in the modified PAM medium contained *E. coli* spheroplasts (approximately 1×10^8 cells, either infected or noninfected); 5 μc each of C^{14}-leucine, C^{14}-valine, and C^{14}-serine; 20 μg of 7,12-DMBA, and 10 μg of actinomycin D. The mixtures were incubated at 30° and 0.2-ml samples were withdrawn at various times, and the incorporation of label into protein was determined as indicated under *Methods*. Phage titer determinations on separate samples, 3 hr after infection, showed a 90% inhibition by 7,12-DMBA.

bon. It is probable that some of the labeled protein represents host protein synthesis, even though actinomycin D was added to the spheroplast mixture. Studies with MS2 antisera suggest a similar hydrocarbon response.

Serum-blocking experiments, as described by De Mars,[10] offer an extremely sensitive method for detecting viral protein synthesis. This procedure involves adding a slight excess of phage antiserum to samples of phage antigen (serum-blocking material), and, after antiserum-antigen interaction, assaying for unreacted antiserum by its ability to inactivate a known quantity of test phage. The extent of test phage survival reflects the amount of unreacted antiserum and provides an indirect measure of the quantity of antigen (viral protein) in the unknown samples. Table 3 shows that in the absence of hydrocarbon, or in the presence of benz(a)anthracene, a sufficient amount of viral protein is made 60 min after MS2 infection, which almost completely "blocks" the inactivation of test phage by the antiserum. In the presence of 7,12-DMBA, no appreciable phage antigen is synthesized 60 min after infection since the test phage is inactivated to nearly the same extent as the controls (zero time) where no phage antigen is present.[11] Similar results were obtained when the cells were lysed 2 hr after infection.

The effect of aromatics on the replication of double-stranded DNA viruses: The "CA method" of preparing infected spheroplasts allows an examination of the hydrocarbon response to double-stranded as well as single-stranded viral replication. If the inhibition of bacteriophage replication by aromatics is a general phenomenon, one would expect the replication of all viruses to be similarly affected. Table 4 shows the effect of the hydrocarbons previously examined on the replication of the double-stranded bacteriophages λ and T4. The results indicate that some of the aromatics that were strong inhibitors for the single-stranded viruses now appear to be either less effective (i.e., 7,12-DMBA and 1,12-DMBA), or show little significant activity (i.e., 6,8-DMBA and 6-MBA). The previous correlation between hydrocarbons active as viral inhibitors and those active as carcinogens no longer seems to hold when the double-stranded viruses are employed as the infecting agents.

The influence of aromatics on host nucleic acid and protein synthesis: In a previous communication,[1] we indicated that the aromatics exerted no observable ef-

TABLE 3

EFFECT OF HYDROCARBONS ON THE PRODUCTION OF SERUM-BLOCKING MATERIAL BY MS2-INFECTED CELLS

Serum-blocking material	MS2 antiserum	Test MS2	Test Phage Survival — PFU	Test Phage Survival — Per cent inactivated
None	0	+	739	0
None	+	+	9	99
No hydrocarbon (0′ lysate)	+	+	11	99
No hydrocarbon (60′ lysate)	+	+	626	15
BA (0′ lysate)	+	+	9	99
BA (60′ lysate)	+	+	572	23
7,12-DMBA (0′ lysate)	+	+	15	98
7,12-DMBA (60′ lysate)	+	+	91	88

MS2 antiserum was prepared from rabbits which had been injected with whole MS2 phage for a period of 1 month. The K value for the stock antiserum (velocity constant for virus inactivation at 37°) was 1.5×10^4 per min. MS2-infected spheroplasts (CA method), after incubation in the absence or presence of hydrocarbon for 60 min, were lysed by freezing and thawing. The lysed cells were exposed to ultraviolet light for 5 hr to inactivate virus; no significant infectivity was detected after this treatment. The ultraviolet-treated lysate (serum-blocking material) was incubated for 12 hr with MS2 atiserum (final concentration 5×10^{-4} dilution of stock), test MS2 phage was added and incubated for another 60 min, and then assayed for surviving virus.

TABLE 4

EFFECT OF VARIOUS HYDROCARBONS ON PHAGE λ AND T4 PRODUCTION

Hydrocarbon	Phage λ		T4		Biological* activity in rats
	Phage titer (PFU/ml × 10^{-5})	Per cent inhibition	Phage titer (PFU × 10^{-7})	Per cent inhibition	
None	88	0	22	0	−
BA	85	3	24	0	−
7,12-DMBA	39	56	12	45	+
6,8-DMBA	90	0	20	9	+
1,12-DMBA	50	43	17	23	−
3,9-DMBA	84	5	15	32	−
4-MBA	77	12	18	18	−
5-MBA	89	0	20	9	−
6-MBA	66	25	19	14	+
Chrysene	79	10	23	0	−

Spheroplasts infected with λ and T4 phage were prepared by the CA method. The assay mixtures and conditions were the same as indicated in Table 1, except that the incubation time of the infectious hydrocarbon mixtures were for 2 hr.

* +, Produces sarcoma when 2.5 mg of hydrocarbon in 0.5 ml of sesame oil is injected in muscle of rat.

fects on nucleic acid and protein synthesis in *E. coli* spheroplasts. Since the assembly of viral constituents involves the use of the host's biochemical machinery, it seemed important to confirm these findings. When spheroplasts were used immediately after preparation, instead of after standing for a few hours as in the earlier studies, 7,12-DMBA (but not benz(a)anthracene) significantly lowered C^{14}-uracil and C^{14}-leucine incorporation into an acid-precipitable fraction (Fig. 4). Many different experiments with *E. coli* spheroplasts now confirm this observation and can be summarized as follows: (1) the active aromatics that inhibit bacterial nucleic acid and protein synthesis appear to be the same as those that inhibit virus formation; (2) the presence of chloramphenicol does not diminish the 7,12-DMBA inhibition of uracil incorporation into RNA, suggesting that this effect is independent of bacterial protein synthesis; (3) 7,12-DMBA inhibits the incorporation of Pi^{32} into acid-soluble organic phosphate (charcoal-adsorbable) and into RNA and DNA; and (4) 7,12-DMBA inhibits the incorporation of various amino acids as well as leucine (e.g., lysine, isoleucine, and phenylalanine) into protein, although there is little or no change in serine incorporation. On occasion, 7,12-DMBA has shown marked inhibiting effects on the polyuridylate-directed assembly of C^{14}-phenylalanine polypeptides in cell-free protein-synthesizing systems with *E. coli* extracts, although this effect has not been consistently reproducible.

Discussion.—The present study confirms and extends some of our earlier observations but also confronts us with some perplexing problems. The two infectious procedures used for the hydrocarbon assay, (*a*) spheroplast infection with viral nucleic acids[1] and (*b*) the conversion of infected bacteria to spheroplasts, show similar inhibitions with the same aromatics for cells infected with single-stranded viruses. Some differences in the sensitivity to certain hydrocarbons have been observed, e.g., the aromatics 3-aminochrysene, benzo(a)pyrene, and 3-methylcholanthrene exhibit stronger inhibitory responses when assayed by the infectious nucleic acid procedure rather than by the "CA method." When spheroplasts infected with the single-stranded viruses are employed, a striking correlation is observed between those aromatics that inhibit viral multiplication and those that induce tumors in rats. The converse correlation for the inactive hydrocarbons is also impressive. Since hydroxylated derivatives of DMBA have been the subject of some attention

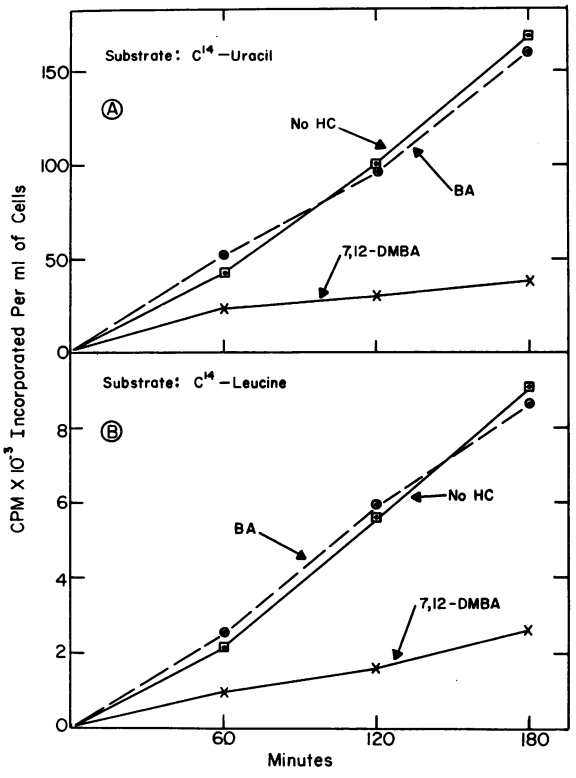

FIG. 4.—The effect of aromatics on *E. coli* spheroplast synthesis of RNA and protein. The reaction mixture contained 1 ml of *E. coli* spheroplasts (1×10^8 cells, noninfected), 20 μg of hydrocarbon, and either 0.01 mc of uracil-2-C^{14} (45 mc per mmole) or 0.01 mc of C^{14}-leucine (222 mc per mmole). The mixture was incubated at 30° and at various times 0.2-ml samples were withdrawn and the label content of the acid-precipitable fraction was determined as described under *Methods*.

in mammalian studies,[12] it might be mentioned that under assay conditions where 7,12-DMBA inhibits MS2 replication by more than 90 per cent, the aromatics 7,12-dihydroxymethyl-BA and 7-hydroxymethyl-12-methyl-BA are without effect, and 12-hydroxymethyl-7-methyl-BA inhibits replication by only 30 per cent. These results are in agreement with the mammalian work where the hydroxymethyl aromatics were found to be less carcinogenic than DMBA. Although the close relationship between hydrocarbon action in the bacterial and mammalian systems may be fortuitous, it is interesting that the viral and mammalian studies show that certain aromatics similarly protect against 7,12-DMBA-induced activity.[9]

Our studies with labeled precursors and MS2 antiserum suggest that hydrocarbon inhibition of MS2 replication involves a reduction in the rate and extent of viral nucleic acid and protein synthesis. The reduced level of viral RNA polymerase in the presence of 7,12-DMBA offers a possible explanation for the lower rate of MS2 RNA synthesis, although the mechanism by which the hydrocarbon reduces this activity cannot be ascertained from these experiments. The net result of the hydrocarbon action appears to be an inhibition in the number of infected cells producing virus, and a lowering of the viral burst size for those cells that do produce virus. It should be pointed out that our assays for *de novo* virus synthesis detect only those viruses that are infectious; defective viruses would not be scored. Since a large number of infected spheroplasts appear to produce no phage in the presence of active hydrocarbons, the question may be raised whether 7,12-DMBA is lethal to the host cell. Titration experiments with penicillin spheroplasts, which are also sensitive to 7,12-DMBA and which can revert to whole cells after removal of the antibiotic, show no detectable decrease in potential colony formers in the presence of 7,12-DMBA.

In view of our present finding that the active hydrocarbons also reduce the efficiency of the host's biochemical machinery to synthesize nucleotides, nucleic acids,

and proteins, the viral inhibition might simply be a reflection of the reduced capacity of *E. coli* cells to manufacture these metabolites in general. Although this explanation is the easiest to accept, it does not account for our results with the double-stranded viruses T4 and λ. If the primary action of the active aromatics is on the host, then the replication of all viruses should be similarly affected; the aromatics inhibitory for MS2 and φX174 replication, however, show reduced or little inhibitory activity on T4 and λ replication. De Maeyer *et al.* have reported that carcinogenic hydrocarbons inhibit the replication of the double-stranded DNA mammalian viruses, herpes, and vaccinia, in rat embryo cultures,[13] yet these workers could demonstrate no inhibition by the same carcinogens on a mammalian RNA-containing virus, Sindbis. Basic differences may exist between the hydrocarbon response of the microbial and mammalian viral systems. At present, our studies offer no explanation for the differences in hydrocarbon response between the single- and double-stranded viruses, but the differences in replication mechanism of these two types of viruses may allow one to observe more definitive effects in one case than in the other. If this were true, it would imply that the active aromatics exert their influence at multiple loci in infected bacteria, at sites sensitive to the operation of the host's metabolic machinery as well as at sites unique to the viral replicative process.

Summary.—A method is described for preparing infected spheroplasts from cells preinfected with phage for use in testing the hydrocarbon effect on viral replication. In cells infected with single-stranded viruses, a close correlation is observed between aromatics that inhibit virus replication and those that are carcinogenic. This relationship, however, is not apparent when double-stranded viruses are used in the same test system. The powerful carcinogen, 7,12-dimethyl-benz(a)anthracene inhibits nucleic acid and protein synthesis specific for viral replication as well as the same syntheses in noninfected cells. Viral replication can be partially protected from 7,12-DMBA by the presence of other aromatics which are not themselves viral inhibitors or carcinogenic inducers.

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† Operated by the University of Chicago for the U.S. Atomic Energy Commission.

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[2] The following abbreviations are used: RNA and DNA for ribo- and deoxyribonucleic acid; DNase, deoxyribonuclease; EDTA, disodium ethylenediamine-tetraacetate; PFU, plaque-forming unit; HC, hydrocarbon; BA, benz(a)anthracene; MBA, methylbenz(a)anthracene; DMBA, dimethylbenz(a)anthracene.

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[11] Since whole-phage antiserum may not interact with phage protein subunits unless they are assembled into a structure comparable to that found in the intact phage, this experiment does not prove that 7,12-DMBA inhibits phage protein formation; however, it is consistent with the amino acid isotope incorporation data.

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