forms, and the tunneling rate. These data are listed in Table 1 for the ground state, the lowest charge-transfer excited state, and the ground states of the anion and cation of the G–C base pair. The tunneling time, τ, is that needed for the departure from tunneling equilibrium to be reduced to $1/e$ of its original value; the equilibrium constant K is the ratio of the populations of the two tautomeric forms.

The data of Table 1 confirm the notion that the probability of an isolated proton rearrangement in the ground electronic state is negligibly small. However, appreciable equilibrium populations of the rare tautomeric form are estimated for both the ionic and excited states, thereby permitting such states to provide opportunities for mutation or carcinogenesis. The effect is most marked for the anion, where the rare tautomeric form is the favored arrangement. However, the cation and excited state both have a sufficiently high rare tautomer population to yield significant mutation rates.

The table also indicates that the proton tunneling is possible from a kinetic point of view. The times required to establish equilibrium are small relative to lifetimes which may be expected for the ions and excited state (which consists of both singlet and triplet spin states whose energy difference was ignored in the present study, and of which at least the triplet would have an appreciable lifetime). Thus, G–C pairs which are not in the ground state of the neutral species at the replication time would have sufficient time to reach the tautomeric equilibrium.

Finally, let us note that the states facilitating proton rearrangements may be reached by the action of radiation in several ways. For example, negative ions could be produced by the capture of free electrons which may be present in irradiated systems. Nonradiative processes, such as donor-acceptor interactions between DNA and chemical mutagens, may also be involved. Such processes might in particular facilitate the formation of the ionic states we have discussed.

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Effects of Polycyclic Aromatic Carcinogens on Viral Replication: Similarity to Actinomycin D

Abstract. *When incorporated into a nutrient overlay, the carcinogenic hydrocarbons benzo*[a]*pyrene and 7,12-dimethylbenz*[a]*anthracene inhibit plaque formation by herpes virus and vaccinia virus, DNA viruses, but not by Sindbis virus, an RNA virus. These carcinogens also decrease herpes and vaccinia virus yields in liquid medium, without affecting Sindbis virus yields. Four structurally related, but noncarcinogenic polycyclic hydrocarbons, namely benzo*[e]*pyrene, pyrene, benz*[a]*anthracene and anthracene, have no inhibitory effect on DNA virus replication. Taken together with the known inhibition of interferon production, these effects on virus growth resemble the action of actinomycin D and hence provide evidence for a selective interaction of these carcinogens with DNA.*

The polycyclic aromatic carcinogens 3-methylcholanthrene, 7,12-dimethylbenz[*a*]anthracene (DMBA), and benzo[*a*]pyrene (BaP) (*1*) inhibit interferon synthesis in tissue cultures of rat cells infected with Sindbis virus (*2*, *3*, and an unpublished observation). Similarly, the antibiotic actinomycin D prevents formation of interferon in cells infected with an RNA virus (*4*). The latter finding suggests that the synthesis of interferon is DNA-dependent and consequently coded by the genome of the cell—not by the virus. Actinomycin D exerts its activity by forming complexes with DNA, thereby arresting the synthesis of DNA-dependent RNA (*5*). This explains why growth of DNA viruses is actinomycin-sensitive, while that of most RNA viruses is not (*5*, *6*). The similarity of effect of actinomycin D and polycyclic carcinogens on interferon synthesis suggested that carcinogens might combine selectively with the DNA of the cellular genome, thus preventing synthesis of interferon, a cellular protein. If this were true, carcinogens, like actinomycin D, should inhibit replication of DNA viruses but not of RNA viruses. This hypothesis was tested by examining the influence of BaP and DMBA on the replication of vaccinia and herpes simplex viruses, DNA viruses, and Sindbis virus, an RNA virus. Structurally related but noncarcinogenic aromatic hydrocarbons were included in the experiment as controls.

The effects of DMBA, benzo[*a*]anthracene (BA), and anthracene, and of BaP, benzo[*e*]pyrene (BeP), and pyrene on plaque formation by vaccinia, herpes simplex, and Sindbis viruses were examined. Both DMBA and BaP are potent carcinogens; BeP, pyrene, and anthracene are not carcinogenic. According to many reports BA is not carcinogenic; according to others, it is a weak carcinogen (*7*, *8*). The compounds were diluted in the culture medium from a stock solution in acetone at 5000 μg/ml; in this way, a very fine suspension in the culture medium was obtained. The plaque assays were carried out as follows. Secondary cultures of rat-embryo cells were grown in plastic petri dishes, 55 or 85 mm in diameter. Before virus inoculation, the growth medium was removed, and 0.9 ml of an appropriate virus dilution was added to each culture. The inoculum fluid was removed after 1 hour, thus removing all unadsorbed virus. A nutrient overlay solidified with starch (*9*) was then added to each culture. This overlay contained the various hydrocarbons at various concentrations (Table 1). The cultures were then kept in an atmosphere of 5 percent CO_2 in air at 36.5°C for 3 to 5 days. Neutral red was added on the morning of the day the plaques were to be counted, 6 to 8 hours before the counts were made. The results of representative experiments are summarized in Table 1.

Plaque formation by the two DNA viruses was significantly inhibited by the carcinogenic compounds, but not by their noncarcinogenic counterparts. Plaque formation by Sindbis virus was unaffected by the carcinogens or by control compounds. Plaque numbers of

the DNA viruses were reduced only when the carcinogens were incorporated into the overlay and were therefore present during viral replication. Incubation of cultures with the carcinogens prior to inoculation of the virus, without subsequent incorporation of the compounds in the overlay, did not significantly decrease the number of plaques. However, the average plaque size was increased (*10*); there is evidence that increased plaque size was due to decreased interferon synthesis.

These results were extended by comparing the influence of the carcinogens and related compounds on the yields of vaccinia, herpes simplex, and Sindbis viruses in rat-embryo cell cultures infected at a high multiplicity with these viruses (Table 2). The growth medium (*2*) of rat-embryo cell cultures in rubber-stoppered tubes was replaced by maintenance medium (*2*) containing the various hydrocarbons at the concentrations indicated in the table. Twenty-four hours later the media were removed from all tubes, and the virus inoculum was added at an input multiplicity of approximately 5 plaque-forming units per cell. The virus was left to adsorb to the cells for 60 minutes, after which time the inoculum fluid was removed. Fresh maintenance medium, containing the same hydrocarbons at the same concentrations as before, was then added to each tube. The cultures were further incubated at 36.5°C before freezing at the time of clearcut cytopathic effect in the control tubes; this occurred 12 to 24 hours after seeding, depending on the assay and the virus.

Table 1. Effect of BaP, BeP, and pyrene and of DMBA, BA, and anthracene on plaque formation by vaccinia, herpes simplex, and Sindbis viruses. Each value represents the average plaque number of at least four cultures.

Test compound	Amount (μg/ml)	Plaque number		
		Vaccinia	Herpes	Sindbis
None		380	91	88
BaP	10			90
BaP	1	281	48	80
BaP	0.1	329	77	
BeP	10	379	90	90
BeP	1	363	82	86
Pyrene	10	412	94	81
Pyrene	1	396	83	80
None		107	50	45
DMBA	10		19	52
DMBA	1	30	20	49
DMBA	0.1	58	29	48
DMBA	0.01	105	35	
BA	10	134	50	57
BA	1	117	50	56
BA	0.1	131	49	
Anthracene	10	121	49	51
Anthracene	1	102	48	46
Anthracene	0.1	102	51	

Table 2. Effect of carcinogenic and noncarcinogenic hydrocarbons on the yields of vaccinia, herpes simplex, and Sindbis viruses in rat-embryo cell cultures. Virus titers are expressed as plaque-forming units per 0.9 ml.

Treatment	Amount (μg/ml)	Virus titer		
		Vaccinia	Herpes	Sindbis
None		1.5×10^5	1.0×10^7	5.8×10^6
BaP	10	2.0×10^4	7.0×10^5	2.1×10^6
BaP	1	1.0×10^4	1.0×10^6	4.9×10^6
BeP	10	1.1×10^5	7.0×10^6	3.5×10^6
BeP	1	1.2×10^5	1.1×10^7	2.9×10^6
Pyrene	10	8.0×10^4	7.8×10^6	2.1×10^6
Pyrene	1	3.0×10^5	2.2×10^7	7.3×10^6
None		6.0×10^5	1.0×10^7	3.0×10^6
DMBA	10	4.5×10^4	9.5×10^5	2.2×10^6
DMBA	1	5.0×10^4	2.3×10^6	5.5×10^6
BA	10	5.5×10^5	1.0×10^7	5.5×10^6
BA	1	1.3×10^5	3.0×10^7	7.0×10^6
Anthracene	10	5.4×10^5	7.2×10^6	2.5×10^6
Anthracene	1	2.1×10^5	1.0×10^7	4.0×10^6

The cytopathic effect in the cases of vaccinia and herpes simplex viruses was clearly diminished in the tubes containing DMBA and BaP, but was not completely inhibited. Before titration of virus yields, intracellular virus was freed into the medium by three cycles of rapid freezing and thawing; the titers given in the table thus represent the combination of intra- and extracellular virus present in the tubes at the time of freezing. The DMBA and BaP decreased the yield of vaccinia virus and herpes virus, but failed to influence the yield of Sindbis virus. The noncarcinogenic hydrocarbons did not affect replication of any of the three viruses.

Selective inhibition of DNA virus replication and suppression of interferon synthesis, at doses of carcinogenic hydrocarbons which do not influence RNA virus growth, closely resemble the effects of actinomycin D under similar conditions. These observations suggest that BaP and DMBA, like actinomycin D, can prevent the expression of genetic information, most probably by combining with DNA, either cellular or viral. The binding of carcinogen to DNA is not purely hypothetical; evidence for the combination of aromatic carcinogens with purified DNA has been obtained in vitro by Boyland and Green (*11*). Furthermore, using labeled compounds, Brookes and Lawley (*12*) recently demonstrated a direct relation between the carcinogenic power of hydrocarbons and their binding in vivo to mouse skin DNA. Binding to RNA also occurred, but at a much lower level.

The results of the present study suggest that the carcinogenic hydrocarbons DMBA and BaP interact with DNA and impair the expression of information contained in this DNA. This effect may be specific, as these compounds had no effect on the multiplication of the RNA virus, and the noncarcinogenic BeP, pyrene, BA, and anthracene showed no activity against the three viruses studied. The influence of carcinogenic hydrocarbons on replication of mammalian viruses is of interest in the study of the effects of these compounds on genetic mechanisms.

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References and Notes

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