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EFFECT OF MITOMYCIN C ON THE GROWTH OF SOME ANIMAL VIRUSES*

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Mitomycin C is a specific inhibitor of DNA biosynthesis in bacterial and mammalian cells.[1,2]   The suppression of DNA synthesis by Mitomycin results from destruction of the DNA-template in both mammalian and bacterial systems.[2-4]   Thus the effect of Mitomycin on DNA is analogous to those of x-rays and the decay of incorporated P^{32}.   Only at very high antibiotic concentrations is the synthesis of RNA and protein affected.   The enzymatic synthesis of DNA *per se* is not inhibited by Mitomycin.   This follows from the fact that bacteria remain capable of supporting the growth of temperate and virulent bacteriophages in the presence of Mitomycin.[5,6]

The possibility of selectively destroying biologically active host-DNA provides a unique opportunity for investigating certain genetic and physiological properties of virus-infected cells.   Much is known of the interactions between bacteriophages and their hosts,[7] but comparable relationships have not been so clearly described for animal viruses.   We have therefore examined the growth of an RNA virus, Mengo encephalomyelitis virus, and a DNA virus, vaccinia, in mammalian cells either in the presence of, or after pre-treatment with, suitable concentrations of Mitomycin C.

Materials and Methods.—Cells: All experiments were conducted with monolayers of L-cells (strain 929), in plastic Petri dishes (60 × 15 mm) containing 5×10^5 cells.   The experiments were begun 16 to 20 hours after plating trypsinized suspensions of cells.   Cells were maintained and propagated by methods previously described.[4]   Monolayers which had been exposed to Mitomycin prior to infection were usually incubated for 16 hours after removal of the compound and before the addition of virus.   Companion cultures, which had not been exposed to Mitomycin, served as controls for all experimental manipulations.   Other cultures, with cells seeded on cover slips in Petri plates, were used for autoradiography to determine the uptake of appropriate radioactive precursors for nucleic acids in each situation under observation.   Thus autoradiographs were obtained on Mitomycin-treated and -untreated cells, both infected with virus and uninfected.

Radioautography: Following incubation in the presence of radioactive thymidine (0.5 μc/ml, usually for 10 hours) or cytidine (0.5 μc/ml, 3 to 6 hours), the cover

slips were removed from the incubation medium and prepared for autoradiography by the method of Doniach and Pelc.[8]   Acid-soluble nucleotides were extracted with 2 per cent perchloric acid at 4°C for 60 minutes.   Cytidine-labeled DNA was removed, when necessary, with DNase.   The film was developed after 3 to 4 days' exposure at 4°C.   All radioactive materials were obtained from New England Nuclear Corp., Boston, Mass.

Viruses: The origin and properties of the Mengovirus used have been described.[9] Vaccinia virus (obtained through the courtesy of Dr. S. Dales) was derived from a strain adapted to grow in L-cells.[10]   All viruses were assayed by the plaque technique.[9]   Infection of L-cells was performed at a multiplicity so that all cells were infected.[11]

Results.—(a) *Mengovirus:*   The results of two representative experiments are shown in Table 1.   The growth of Mengovirus was not greatly inhibited by any of

TABLE 1

EFFECT OF MITOMYCIN C ON MENGOVIRUS MULTIPLICATION*

Experiments	Dose of Mitomycin C, γ/ml	PFU/cell
No. 1	0	570
	5	470
	15	500
	30	420
No. 2	0	2950
	5	2030
	15	540
	30	530

* Cells were exposed to Mitomycin for 8 hr, and then incubated for 16 hr in Mitomycin-free medium prior to infection with Mengovirus.   Infected cultures were incubated in Mitomycin-free medium for 12 hr.

the concentrations of Mitomycin tested.   Although very high concentrations (30 to 50 γ/ml) may depress virus yields as much as 80 per cent, this finding has been variable, and virus yields equivalent to those seen in control cultures have been observed at such concentrations of Mitomycin (Table 3).

TABLE 2

EFFECT OF VARIATIONS IN THE EXPOSURE OF L-CELLS TO MITOMYCIN C ON THE GROWTH OF MENGOVIRUS

A.   Actively dividing cells were exposed to Mitomycin for 8 hr, and then incubated for 16 hr in Mitomycin-free medium.   At this time the cells were infected with Mengovirus and virus allowed to multiply in the absence of Mitomycin.   Control cells were infected at the beginning of the period of Mitomycin treatment.   During the eclipse phase there was an average of 0.5 PFU/cell.

Concentration of Mitomycin, γ/ml	PFU/cell, 12-hr yield
0	850
5	690
15	360
30	300

B.   Mitomycin was added to the input virus and therefore cells were exposed to the drug during adsorption. Mitomycin was also present in the growth medium, i.e., throughout the period of virus growth.   The host cells were not exposed to Mitomycin prior to the addition of virus.   The control was the same as in part A.

Concentration of Mitomycin, γ/ml	PFU/cell, 12-hr yield
5	1400
15	1100
30	1000

The data shown in Table 1 were obtained under conditions in which the host cells had been exposed to Mitomycin prior to the addition of virus.   The presence of Mitomycin throughout the experiment did not affect the results, however, as is seen from the data in Table 2.

TABLE 3

EFFECTS OF HIGH DOSES OF MITOMYCIN C ON THE GROWTH OF MENGOVIRUS IN L-CELLS

Concentration of Mitomycin and Treatment		PFU/cell
0	0 time	5.8
0	24-hr yield	750
30 γ/ml	Present throughout	580
50 γ/ml	Present throughout	920
50 γ/ml	8-hr pulse, infection immediately after pulse	1,310
50 γ/ml	8-hr pulse, infection 16 hr after pulse	230

Virus yields from Mengovirus-infected L-cells vary over a wide range.[11]   This may reflect physiological conditions which vary in the cellular population in different experiments.   Exponentially growing host cells produce virus yields considerably greater than do stationary cultures.   The lower yields of virus sometimes observed in cells which had been pretreated with very high concentrations of Mitomycin may, therefore, be attributable to the 16-hour period of incubation following Mitomycin treatment.   During this period, cells whose DNA content had been badly damaged may fail to renew their enzyme systems, thereby decreasing the concentration of intracellular metabolites necessary for virus multiplication so that the reduced capacity for virus production in such cells may be basically metabolic rather than genetic.   For this reason experiments were performed in which cells exposed to Mitomycin for 2 hours were infected with Mengovirus in the presence of the antibiotic, and exposure to the drug was continued throughout the viral growth period.   The virus yield of such cells was compared with that obtained from others treated with Mitomycin for the usual 8-hour period and infected immediately, or 16 hours after, the termination of Mitomycin exposure.   The antibiotic concentrations used were those known to inhibit DNA synthesis (30 γ/ml) as well as RNA synthesis (50 γ/ml).   The results are shown in Table 3.

Pulses of Mitomycin C at concentrations above 15 γ/ml for periods of at least 8 hours appear to suppress subsequent DNA synthesis completely, within the limits of resolution of the autoradiographic technique.   Following incubation in thymidine-H³, cells treated in this way showed no radioactivity above background.   In our hands this technique would detect a degree of DNA synthesis equal to 0.1–0.2 per cent of normal during the 10-hour period under examination.   Thus these cells apparently possessed no DNA capable of replication and therefore, it follows that the production of viral RNA and virus-specific protein does not require the presence of biologically intact, replicating cellular DNA.

The inhibition of cellular DNA synthesis by Mitomycin is not measurably reversed by Mengovirus infection.   Indeed, at 15 γ Mitomycin/ml for 8 hours the slight residual incorporation of thymidine into DNA sometimes found in uninfected cells is significantly inhibited in the infected cell.   *Thus, in the case of Mengovirus, viral RNA synthesis does not require an associated or preliminary synthesis of DNA, nor does it require the integrity of any particular host genome.*

(b) *Vaccinia:*   The experiments of Cairns on the initiation of vaccinia infection suggested that the host-virus relationships in this case might be comparable in some respects to those found for T-2 bacteriophage infection in *Escherichia coli.*[12]   For this reason, the data shown in Table 4 are of particular interest.   A profound inhibition of vaccinia growth is caused by Mitomycin, either through pre-treatment of the host cell or by its presence throughout the course of infection.   *In vitro*

TABLE 4
EFFECT OF MITOMYCIN C ON THE GROWTH OF VACCINIA VIRUS

Concentration of Mitomycin, γ/ml		Yield in PFU/cell, cells pretreated with Mitomycin		Yield in PFU/cell, cells in Mitomycin throughout	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2
0	0 time	0.23	0.64	0.23	0.64
0	24-hr yield	18	60	18	60
1	24-hr yield	...	124	...	32
5	24-hr yield	1.2	3.8	1.8	0.72
15	24-hr yield	1.8	1.3	...	...
30	24-hr yield	0.03	1.3	0.01	0.1

The procedure used in these experiments was exactly as in experiments with Mengovirus described in Table 3.

TABLE 5
EFFECT OF MITOMYCIN C ON VACCINIA VIRUS INFECTIVITY

	PFU/ml after 4-hr incubation	PFU/ml after 24-hr incubation
Control	1.14×10^6	5.99×10^5
Mitomycin	1.48×10^6	4.32×10^5

Vaccinia, in Eagle's minimal essential medium with 2 per cent fetal calf serum, was incubated with Mitomycin C at 25°C in the dark. The final concentration of Mitomycin was 10 γ/ml.

incubation of the virus with Mitomycin did not affect its infectivity (Table 5).

The synthesis of DNA peculiar to vaccinia-infected cells can be demonstrated using tritiated thymidine.[12] The label is taken up into acid-insoluble material in the cytoplasm, rather than in the nucleus. Cells were exposed to doses of 5 or 15 γ Mitomycin/ml for 8 hours and then immediately infected with vaccinia. Under these conditions there was no virus synthesis. Cells were exposed to tritiated thymidine from 6 to 8 hours after the start of infection. Uptake of H^3-thymidine into nuclear DNA is reduced to 1–10 per cent of the normal level in both infected and noninfected cells. At 5 γ Mitomycin/ml there is uptake of label into cytoplasmic DNA in only a few cells and at 15 γ/ml there is no uptake into cytoplasmic DNA. Thus Mitomycin prevents synthesis of virus and viral DNA in this case.

The observations discussed above together with those found in cells treated with Actinomycin,[13] suggest that vaccinia growth is indeed related to, and dependent on, nuclear events in the host cell. *A substantially intact host genome and a normally functioning cellular pathway of genetic expression appear to be required for normal vaccinia growth, at least in L-cells.*

Discussion.—The *in vivo* growth of DNA and RNA viruses in neurons of the adult mammalian central nervous system has made it clear that the production of viral DNA and RNA does not require active cellular DNA synthesis. Simon showed that the multiplication of RNA viruses does not depend on a concomitant synthesis of cellular DNA.[14] In these studies inhibitors which prevented DNA synthesis in cultured host cells were used.

The findings reported here and those of Simon suggest that the presence of *biologically intact DNA* is not required for normal RNA virus growth and nucleic acid synthesis. This is noteworthy since the highest level of Mitomycin employed (50 γ/ml for 8 hours) suppresses cellular RNA synthesis as well as DNA synthesis. This finding is not surprising since it may be presumed that most, if not all, cellular RNA synthesis is under genetic control and not independent of DNA. That viral RNA can be replicated in cells which, due to destruction of their genome, no longer are capable of synthesizing their own specific RNA appears to place viral RNA

synthesis in a category different from that of cellular RNA synthesis. Normal DNA-dependent cellular RNA synthesis may represent an enzymatic process which does not necessarily contribute to the production of viral RNA in the infected cell.

Ben-Porat *et al.* reported the production of noninfectious pseudorabies particles in Mitomycin-treated rabbit kidney cells.[15] It is not possible to correlate their results completely with the observations reported here, since the experimental conditions used by Ben-Porat *et al.* probably did not totally destroy the host genome, and their study of virus infection did not include pretreatment of cells with Mitomycin followed by infection in Mitomycin-free medium. Some dependence of synthesis of viral subunits on intactness of host genome can, therefore, not be excluded in this case. Their results suggest that viral DNA synthesis and the expression of at least some viral genetic potentialities could occur in the presence of high, if not totally destructive, concentrations of Mitomycin. This finding resembles that reported for bacteriophages[5, 6] whose growth is not inhibited by Mitomycin. Since the rate of depolymerization of DNA by Mitomycin is slow in relation to the rate of DNA synthesis in phage-infected cells,[4] viral growth and expression of viral genetic traits may occur in the presence of Mitomycin. Thus we conclude that the time required for assembly of mature virus from its newly synthesized constituents permits the destructive action of Mitomycin to be exerted on the genetic material of the particles finally produced. This results in a large proportion of the new particles being noninfectious, presumably due to some interruption in the continuity of their DNA. The parallelism of these phenomena is striking in two DNA viruses, one a bacterial[4] and the other an animal[13] virus.

The results reported in this paper, that in Mengovirus infection viral RNA and protein synthesis are unaffected by Mitomycin, taken with those of Ben-Porat *et al.* on pseudorabies virus[15] and Sekiguchi *et al.* on T-phages,[6] emphasize the highly specific cytocidal action of the antibiotic, based on the destruction of DNA. RNA biosynthesis is affected only to the extent that destruction of the cellular genome removes the governing templates. Introduction of viral DNA into a cell whose genetic apparatus has been badly injured by Mitomycin permits the formation of whatever new RNA may be required for virus-specific protein synthesis. Where genetic specificity resides in RNA, no DNA appears to be required for RNA production.

Cairns has reported[12] that viral DNA synthesis in cells infected with vaccinia could proceed in the cytoplasm and concluded that the virus was virulent and independent of its host. Our data may implicate nuclear participation at some stage in vaccinia growth, presumably that involving synthesis of viral DNA. Mitomycin-treated cells infected with vaccinia showed no uptake of tritiated thymidine. Therefore, the effect of Mitomycin on the growth of this pox virus presumably affects an early stage in its development, and correlates with the absence of the characteristic cytopathic effect which this virus strain normally produces in L-cells.

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MANY-ELECTRON THEORY OF ATOMS AND MOLECULES

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Orbital theories of atoms and molecules work quite well in a qualitative or semi-empirical way but fail when put to a quantitative test. The error, which is so large that often even molecule formation is not predicted, is generally attributed to electron correlation. Its importance has been stressed in several reviews.[1, 2]

Since electrons affect one another through their instantaneous potentials and not just by their average potentials as in the Hartree-Fock method,[1] we do have a many-electron problem. But what sort of a many-body problem? Does the long range of coulomb repulsions cause all electrons to be involved in one complicated motion? If this were the case to the extent of overcoming the effects of the exclusion principle, shell structure would be wiped out, and an atom or molecule would be more like a drop of electron liquid. Actual shell structure and electron densities are close to those given by the Hartree-Fock method. For example, densities from the latter agree quite well with X-ray results.[3]

The situation of the many-electron problem in atoms and molecules is compared with related many-body problems in Figure 1. Inside nuclei, strong, short-range forces cause only local nucleon pair correlations to dominate. Brueckner theory[4] considers these for the idealized infinite nuclear "matter." Due to the strength of repulsions, each particle moves in an environment it is constantly polarizing. In finite systems,[5] difficulties arise. This "polarized sea" potential is strongly dependent on particle state, so that orthogonal ground-state orbitals cannot be obtained easily. The basis is discrete, and the difficulties of evaluating slowly convergent infinite sums as in ordinary perturbation theory[1] appear.

In the infinite electron gas, Hartree-Fock orbitals being plane waves, there is no electron localization. As we shall see below, correlation is determined by the difference of the instantaneous coulomb potential $g_{ij} = r_{ij}^{-1}$ between two electrons i, j and the average, i.e., Hartree-Fock (H.F.), potential they would exert on one