on a very limited study, it does suggest that the simian reservoir is probably of limited significance in the Philippines for the following reasons: (i) *Plasmodium malariae*, which bears the closest resemblance to *P. inui*, was identified in only 26 out of a total of 41,945 positive human blood smears between 1 July 1959 and 30 June 1960. (ii) In frontier settlements, the species of malaria are divided almost equally between *P. falciparum* and *P. vivax*, which are both morphologically dissimilar to *P. inui*. (iii) In the northern Philippines (Luzon), the pattern of malaria parallels the pattern in the southern islands without evidence, so far, that simian malaria is present.

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References

1. D. E. Eyles, R. G. Coatney, M. E. Getz, *Science* **131**, 1812 (1960).
2. J. A. Sinton, *Records Malaria Survey India* **4**, 379 (1934); H. W. Mulligan and C. S. Swaminath, *J. Malaria Inst. India* **3**, 603 (1940); S. Inoki, *Med. J. Osaka Univ.* **2**, 45 (1951); Garnham, *Riv. parassitol.* **20**, 273 (1959); M. Mayer, *Med. Elin.* **3**, 579 (1907); S. B. de Aberle, *Primate Malaria* (National Research Council, Division of Medical Sciences, Washington, D.C., 1945).
3. A. Mohiuddin, *Rev. de Mal.* **36**, 203 (1957).
4. L. Halberstadter and S. Prowazek, *Arb. Reichsgesundh.* **26**, 1, 37 (1907).

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Effect of Actinomycin D on Cellular Nucleic Acid Synthesis and Virus Production

Abstract. Actinomycin D inhibits the synthesis of ribonucleic acid in L cells and the yield of vaccinia virus containing deoxyribonucleic acid, but it does not inhibit cellular deoxyribonucleic acid synthesis or the multiplication of Mengo virus containing ribonucleic acid. These observations serve to distinguish the replication of viral ribonucleic from ribonucleic acid synthesis which is controlled by viral or cellular deoxyribonucleic acid.

Actinomycin D is a bright red antibiotic containing two peptides, which was first reported by Vining and Waksman (*1*). It possesses strong antibacterial activity against gram-positive organisms (*2*) and, on a weight basis, is the most potent chemotherapeutic antitumor agent known (*3*). It has been reported to be antimutagenic (*4*).

Mammalian cells grown in the presence of actinomycin D lose their nucleoli and much of their histochemically demonstrable ribonucleic acid (RNA) (*5*, *6*). In the present study we report that actinomycin selectively and irreversibly suppresses mammalian cellular RNA biosynthesis, at least up to 48 hours after exposure—the period during which affected cells have been observed.

Strain 929 L-cells monolayers were maintained and propagated as previously described (*6*). In addition, spinner cultures (*7*) were employed. Cell monolayers, seeded on cover slips and exposed to suitable radioactive nucleic acid precursors, were examined autoradiographically by the method of Doniach and Pelc (*8*).

When normal L cells are incubated with H^3-cytidine (0.5 μc, 0.4μg/ml, 3 to 6 hours) and inspected after radioautography, a portion of the radioactivity incorporated into acid-insoluble material may be solubilized by deoxyribonuclease. The remainder can be rendered acid-soluble by digestion with ribonuclease.

After exposure to actinomycin D (1.0 μg/ml for 8 hours) and subsequent incubation for 16 hours, L cells continue to incorporate H^3-cytidine into acid-insoluble material. In this case, however, none of the incorporated radioactivity becomes acid-soluble after ribonuclease treatment; all of it is solubilized by deoxyribonuclease. We conclude that cellular RNA synthesis, but not DNA synthesis, has been completely arrested by antecedent incubation with actinomycin.

Similar findings are shown in Table 1, in which pairs of spinner cultures are compared with respect to the incorporation of H^3-leucine into protein, H^3-uridine into RNA, and H^3-thymidine into DNA 4 hours after initial exposure to actinomycin. One pair of cultures contained actinomycin D (0.2 μg/ml), the other served as control. While the incorporation of leucine into protein and of thymidine into DNA were not affected by the antibiotic, uridine incorporation into RNA was depressed. After 24 hours, uridine uptake into RNA of the cells growing in the presence of actinomycin was still further decreased relative to the control.

The effect of actinomycin on virus growth has also been investigated. In some experiments cells had been treated previously with appropriate concentrations of the antibiotic, while in others actinomycin was present throughout the period of virus absorption and growth. The results were independent of the type of exposure.

Table 1. Incorporaton of precursors into protein, RNA, and DNA*

Compound	Control	Actinomycin treated
Leucine-H^3 into protein	50,980	49,620
Uridine-H^3 into RNA	160,500	66,500
Thymidine-H^3 into DNA	194,000	181,000

* Values expressed as total counts per minute for infinitely thin platings of equal aliquots obtained as follows: one pair of replicate cultures incubated in Eagle's medium containing 0.2 mmole of L-leucine received DL-leucine-H^3 (1 μc/ml) and thymidine-H^3 (0.5 μc, 0.4 μg/ml) and the second pair uridine-H^3 (0.5 μc, 0.8 μg/ml). One culture of each pair was exposed to actinomycin (0.2 μg/ml) for 30 min before and 3½ hours after addition of labeled compounds. Samples of 25 ml each were centrifuged and carriers added; they were then washed with phosphate-buffered saline, extracted with cold 0.25*N* $HClO_4$ for 1 hour, and washed with ethanol and ethanol-ether. For leucine and thymidine determinations the washed pellets were hydrolyzed in 0.5*N* $HClO_4$, washed with ethanol and ethanol-ether and the pellets and hydrolysates were counted. For uridine measurement the pellets were incubated with ribonuclease (150 μg/ml) for 2 hours at 37°C in .005*M* tris buffer, *p*H 8, made 0.2*N* with $HClO_4$, centrifuged, and the supernatant was counted.

The multiplication of vaccinia, a DNA virus, is sensitive to actinomycin, but somewhat less so than division of the host cell: 0.1 μg/ml inhibited vaccinia growth by 99 percent, whereas 0.005 μg/ml suppressed host cell division. On the other hand, concentrations of actinomycin in as high as 10 μg/ml did not inhibit the growth or affect the yield of Mengo virus, a ribonucleic acid virus.

Parallel findings have been obtained with mitomycin, high concentrations of which inhibit cellular but not viral RNA synthesis (*9*). Whereas mitomycin appears to affect RNA synthesis by destroying the genes under whose control the various cellular RNA species are synthesized, actinomycin would seem to leave the genetic apparatus intact since DNA replication is not abolished. This is supported by studies on the effects of actinomycin S on phage synthesis (*10*). The inhibition of T2-phage reproduction by actinomycin does not result in inhibition of DNA synthesis in the phage-infected cell, whereas no phage protein appears to be made. Actinomycin thus appears to block the expression of genetic potentialities by interfering with that portion of RNA synthesis which is dependent on or governed by cellular or viral DNA. This is, therefore, a second line of evidence serving to differentiate replication of viral RNA from that of

cellular RNA. Reduplication of viral RNA is not necessarily inhibited by factors capable of interfering with RNA synthesis which is governed by viral or host DNA. Presumably, therefore, the two RNA synthetic processes are enzymatically or topographically distinct (*11*).

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References and Notes

1. L. C. Vining and S. A. Waksman, *Science* **120**, 389 (1954).
2. L. H. Pugh, E. Katz, S. A. Waksman, *J. Bacteriol.* **72**, 660 (1956).
3. S. Farber, in *Amino Acids and Peptides with Antimetabolic Activity*, Ciba Foundation Symposium, G. E. W. Wolstenholme and C. M. O'Connor, Eds. (Little, Brown, Boston, Mass., 1958), p. 140.
4. W. J. Burdette, *Science* **133**, 40 (1961).
5. D. E. Rounds, Y. H. Nakanishi, C. M. Pomerat, *Antibiotics and Chemotherapy* **10**, 597 (1960); M. N. Goldstein, E. Pfendt, J. D'Arrigo, *Anat. Record* **139**, 231 (1961).
6. A. J. Shatkin, E. Reich, R. M. Franklin, E. L. Tatum, *Biochim. et Biophys. Acta*, in press.
7. W. F. McLimans, E. V. Davis, F. L. Glover, G. W. Rake, *J. Immunol.* **79**, 428 (1957).
8. I. Doniach and S. R. Pelc, *Brit. J. Radiol.* **23**, 184 (1950).
9. E. Reich and R. M. Franklin, *Proc. Natl. Acad. Sci. U.S.*, in press.
10. A. Nakata, M. Sekiguchi, J. Kawamata, *Nature* **189**, 246 (1961).
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Rapid Development of Drug-Resistant Mutants of Poliovirus

Abstract. Guanidine hydrochloride is a potent inhibitor of poliovirus synthesis in cell culture. However, the viral progeny which do grow in the presence of guanidine may become approximately 10,000 times more resistant to the drug. The phenomenon of drug resistance poses yet another problem in the search for a satisfactory viral chemotherapeutic agent.

Because guanidine is a potent inhibitor of poliovirus multiplication in tissue culture (*1, 2*), we tested the drug on monkeys infected orally with poliovirus (*3*). This provided us with virus which had multiplied in vivo in the presence of guanidine. In the present report we wish to call attention to the fact that when virus highly susceptible to the drug is grown in the presence of the drug either in vitro or in vivo, the progeny of the virus become drug resistant.

Resistance produced in vitro. Wild Mahoney poliovirus was carried for five passages in monkey kidney tube cultures in the presence of guanidine hydrochloride; MG_1 and MG_5 are abbreviations for Mahoney passed once and five times, respectively, in the presence of guanidine. Controls passed in aliquots of the same cultures but in the absence of guanidine were labeled MC_1 and MC_5. Increasing concentrations of guanidine from 20 μg/ml to 75 μg/ml (at the fifth passage) were used.

At each passage the viruses were harvested when over 75 percent of the cells were showing pathologic changes, and 0.1 ml was transferred to new cultures. Table 1 shows that virus many thousand times more resistant than the original virus has been obtained by selecting out guanidine-resistant variants. Since the first passage specimen already contained virus which was considerably more resistant than controls, it appears that a selection of spontaneously occurring mutants occurred in the first few cycles of multiplication in the presence of the drug.

Attenuated LSc strain, the very virus used in the oral polio vaccine, was treated in a similar manner. Again the first and fifth passage materials were found to be considerably more resistant to guanidine, the results being similar to those shown in Table 1.

Resistance produced in vivo. Cynomolgus monkeys fed three times daily with guanidine hydrochloride at near toxic levels (60 to 80 mg/kg per day) and fed virulent type 1 Mahoney poliovirus 3 days after the initiation of the drug course developed paralysis about as frequently as monkeys not treated with guanidine (*3*). On the 5th day after virus feeding, which was the 8th day of drug administration, virus was isolated from the blood of two monkeys. The resistance of these viruses to guanidine was compared with that of the original virus. The recovered progeny strains were grown in cultures free of guanidine before their drug resistance was measured.

A plaque titration was done with the above samples with an overlay containing 28 μg of guanidine per milliliter (*2*). One set of controls was set up containing the progeny viruses with normal overlay. A further set of controls included Mahoney virus from the same sample as that used in inoculating the monkeys. The original virus was inhibited 100 to 1000 times more than either of the viruses which had multiplied in the monkeys fed guanidine. Plaques of resistant virus under an overlay of 28 μg of guanidine per milliliter were picked and passed in tube cultures without guanidine. On subsequent titrations this virus was found to be as resistant as before, indicating that the property is stable for several cycles of multiplication in the absence of guanidine.

Table 1. Emergence of mutants of poliovirus resistant to guanidine hydrochloride. PFU, plaque-forming units; MC_1, MC_5, Mahoney virus passed one and five times, respectively, in control cultures without guanidine; MG_1, MG_5, Mahoney virus passed one and five times, respectively, in the presence of guanidine.

Concn. of drug in overlay (μg/ml)	Titers of virus (PFU/ml)			
	MC_1	MG_1	MC_5	MG_5
30	$<10^2$	$10^{5.7}$	$<10^2$	$10^{6.3}$
None	$10^{7.7}$	$10^{7.5}$	$10^{8.0}$	$10^{7.8}$

Comment. Strains of poliovirus have been produced that are over a thousandfold more resistant to guanidine than the original viruses. The mechanism of the resistance is unknown. Nevertheless, the development of resistance to guanidine by poliovirus may be likened to the development of resistance to chemotherapeutic agents by certain bacteria, and it creates a potential difficulty in the development of an efficient viral chemotherapeutic agent.

The production of resistant strains which are easily distinguished from the parent strain offers a useful tool in the study of viral genetics. The fact that drug-resistant strains were as readily selected from progeny of an attentuated vaccine strain as from progeny of a wild strain emphasizes again the genetic pliability of the polioviruses (*4, 5*).

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References and Notes

1. W. A. Rightsel, J. R. Dice, R. J. McAlpine, E. A. Timm, I. W. McLean, Jr., G. J. Dixon, F. M. Schable, Jr., *Science* **134**, 558 (1961).
2. D. Crowther and J. L. Melnick, *Virology*, in press.
3. J. Barrera-Oro and J. L. Melnick, *Texas Repts. Biol. and Med.*, in press.
4. J. L. Melnick and M. Benyesh-Melnick, *Pan-Am. Sanitary Bur. Scientific Publ. No. 50* (1960), p. 12; R. Dulbecco, *ibid.*, p. 47.
5. This study was aided by a grant from the National Foundation and a training grant (2E-74) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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