studies careful removal of barium eliminated this spot and did not significantly alter the remainder of the chromatogram. Simple extraction of thyroid glands with collidine-lutidine revealed in the extracts the presence of radioactive spots corresponding to free iodide and mono- and diiodotyrosine, but no thyroxine was detected. This would indicate that mono- and diiodotyrosine exist in free form and are not completely bound to protein.

References

1. Consden, R., Gordon, A. H., and Martin, A. J. P. *Biochem. J.*, 1944, **38**, 224.
2. Dent, C. E. *Biochem. J.*, 1948, **43**, 169.
3. Fink, R. M., Dent, C. E., and Fink, K. *Nature*, Lond., 1947, **160**, 801.
4. Fink, K. and Fink, R. M. *Science*, 1948, **108**, 358.
5. Hird, F. J. R. and Trikojus, V. M. *Aust. J. Sci.*, 1948, **10**, 185.
6. Williams, R. J. and Kirby, H. *Science*, 1948, **107**, 481.

The Effects of Antagonists on the Multiplication of Vaccinia Virus *in Vitro*

Randall L. Thompson, Marian L. Wilkin, George H. Hitchings, Gertrude B. Elion, Elvira A. Falco, and Peter B. Russell

Department of Microbiology, Indiana University Medical Center, Indianapolis[1] and *The Wellcome Research Laboratories, Tuckahoe, New York*

A large series of analogues og purine and pyrimidine bases and related substances (*6–9*) have been tested for their ability to interfere with the multiplication of vaccinia virus in tissue culture. The method described by Thompson (*11*) measures the multiplication of the virus in the presence and absence of added substances. The inhibitory effects of substituted amino acids (*11*) and of a phenylalanine analogue (*12*) have been reported.

In the general field of substances which might be expected to interfere with nucleic acid synthesis, many substances diminish the rate of multiplication of the virus virus during the incubation period, thus having an ap- and a few bring about a diminution in the titer of the parent virucidal activity.

In many instances the activities of the analogue on vaccinia virus bear a close resemblance to those on *Lactobacillus casei* (*6, 9*). Thus among the substances structurally related to thymine, 5-bromouracil, 5-nitrouracil, dithiothymine, and isobarbituric acid give small but significant and reproducible inhibitions (Table 1). Certain amides of aminouracil, such as 5-*p*-nitrobenzamidouracil, show similar activity. 2,6-Diaminopurine exhibits strong inhibitory effects, which are reversible by purines and nucleic acid derivatives (*13*).

One outstanding difference between vaccinia virus and the bacterial and other growth systems lies in the failure of folic acid antagonists to show more than minimal inhibitions of the virus. This applies not only to the simpler bases, such as 2,4-diamino-5,6-dimethylpyrimidine, which exhibit antifolic activity in the bacterial systems (*8*), but also to the structural analogues of pteroylglutamic acid (PGA) such as 4-aminofolic acid, as shown in Table 1. The inhibitory effects of the structural analogues of PGA are evidenced in many biological systems which involve the rapid proliferation of cells (*2–4*). In general, diaminopurine has been found to have similar effects (*1, 5, 8*). However, a different locus of action of the two antagonists (*6*) is indicated by reversal studies (*6, 8*), and by the hematological findings (*10*). A possible explanation of the present studies might be that the proliferation of the virus occurs via a pathway which is blocked by diaminopurine but not by the folic acid analogues.

TABLE 1

Influence of Pyrimidine Analogues on Multiplication of Vaccinia Virus

Compound	Conc. mg/ml	Increase in virus titer (logarithm 50% end point)	
		Control	Treated
5-Bromouracil	0.1	2.10	1.70
5-Nitrouracil	0.1	2.20	1.80
5-Hydroxyuracil	0.1	2.20	1.36
2,4-Dithiothymine	0.1	1.96	1.48
5-*p*-Nitrobenzamidouracil	0.1	2.30	1.67
2,6-Diaminopurine	0.05	1.30	−0.69
2,4-Diamino-5,6-dimethylpyrimidine	0.05	1.55	1.59
	0.1	1.55	1.14
4-Aminofolic acid	0.1	2.51	1.92

References

1. Burchenal, J. H. *et al.* *Cancer*, 1949, **2**, 119.
2. *Ibid.*, 113.
3. Franklin, A. L. *et al.* *J. biol. Chem.*, 1947, **169**, 427.
4. Hertz, R. *Science*, 1948, **107**, 300.
5. Hertz, R. and Tullner, W. W. *Science*, 1949. Vol. **109**, 139.
6. Hitchings, G. H. *et al.* *Proc. N. Y. Acad. Sci.* In press.
7. Hitchings, G. H., Elion, G. B., and VanderWerff, H. *Fed. Proc.*, 1948, **7**, 160.
8. Hitchings, G. H. *et al.* *J. biol. Chem.*, 1948, **174**, 765.
9. Hitchings, G. H., Falco, E. A., and Sherwood, M. B. *Science*, 1945, **102**, 251.
10. Philips, F. S. and Thiersch, J. B. *Fed. Proc.*, 1949. **8**, 325.
11. Thompson, R. L. *J. Immunol.*, 1947, **55**, 345.
12. Thompson, R. L. and Wilkin, M. L. *Proc. Soc. exp Biol. Med.*, 1948, **68**, 434.
13. Thompson, R. L. *et al.* To be published.

[1] Parts of this program were carried out at Western Reserve University Medical School during 1945 and at the Medical College of Virginia 1946–7.