TABLE I. Effect of Various Amino Acid Deficiencies on Stimulation of Growth of Psittacosis Virus (6BC) in Chick Embryo Tissue Cultures Cultivated in BSS for 13 Days.

Amino acid deficiency		Virus titers* 13th day	14th day	18th day	22nd day
Aspartic acid		1.9	.1	4.7	5.0
Controls	Complete medium	1.9	.1	3.0	5.3
	BSS	1.9	.9	.1	.1
Hydroxyproline		3.1	.5	1.9	4.2
Controls	Complete medium	3.1	.1	2.1	4.2
	BSS	3.1	.1	.1	.1
Lysine		1.9	.1	3.9	5.4
Controls	Complete medium	1.9	.1	3.0	5.4
	BSS	1.9	.9	.1	.1

* Log of LD_{50}.

cellular metabolism into the reduplication of virus particles. Hence, when the virus enters a deficient cell, it remains present in the latent state in the non-infectious phase(2) until such time as the necessary metabolites become available, or until the death of the cell. In psittacosis, a disease characterized by long periods of latent infection in birds, this concept is of special interest, as a variety of changes in the host may lead to the activation of infection. As indicated by these studies, changes in cell nutrition as it relates to the amino acid may be important determinants in such activation.

Summary. Studies on the amino acid requirements for the activation of latent infection with psittacosis virus of chick embryo tissues cultivated *in vitro* revealed that phenylalanine and tryptophane are essential to the proliferation of psittacosis virus, while aspartic acid, hydroxyproline, and lysine are not. The importance of host cell metabolism to viral infection is discussed with special reference to the amino acids.

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Tungstate Antagonism of Molybdate in *Aspergillus niger*.* (22527)

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A requirement for molybdenum by *Aspergillus niger* has been well established(1), particularly when nitrate is the sole source of nitrogen(2). Mo is specifically required in the nutrient medium for the enzymatic reduction of nitrate(3), and Nicholas and Nason (4) showed that it is part of the prosthetic group of the enzyme nitrate reductase. With Mo present in the growth medium, no nitrogen compounds other than the nitrate ion are

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TABLE I. Tungstate Inhibition of Growth of *Aspergillus niger* on a Nitrate Medium.*

	Molybdenum content (mμg)			
μg W added	1.0	2.5	3.5	5.0
0	437	572	683	712
.1	410	510	574	675
.5	228	432	454	562

* mg dry wt of mycelium after 6 days.

required. Tungstate has been shown to be a dietary antagonist of molybdate in animal nutrition(5), and this report presents evidence that tungstate also functions as a competitive inhibitor of molybdate in *Aspergillus niger*.‡

Methods. The microbiological assay for molybdenum, as described by Nicholas(6), was the procedure used in these studies. Each 500 ml pyrex assay flask contained 50 ml of culture solution, freed of Mo by coprecipitation on CuS, plus 10 ml samples of sodium molybdate and/or other salts in appropriate concentrations. After inoculating with *A. niger* spores and incubating for 6 days at 25 ± 0.5°C, the mycelial felts were harvested, washed, dried at 80°C overnight and weighed. When the culture medium was adequate in all nutrients except Mo, and when nitrate was the sole nitrogen source, the dry weight of the mycelium was dependent upon the availability of Mo.

Results. Table I shows that the addition of 0.1 to 0.5 μg of W (as Na_2WO_4) to a culture medium containing 1 to 5 x 10^{-3} μg of Mo (as Na_2MoO_4) depressed the growth of *A. niger*. Two micrograms of tungsten in a system containing 2.5 x 10^{-3} μg Mo per 60 ml prevented growth completely, and this inhibition was overcome entirely by increasing the Mo content to 1 μg (W:Mo ratio of 1:1). A W:Mo ratio of unity maintained normal growth at all levels tested, up to the highest W content of 10 μg per 60 ml. Molybdate concentrations in the nitrate medium were varied from 1.74 x 10^{-10} M to 8.69 x 10^{-10} M (1 to 5 mμg Mo per 60 ml), and the typical Lineweaver-Burk(7) plots of competitive

‡ The *Aspergillus niger* (Mulder strain) used in these studies was generously supplied by Dr. D. J. D. Nicholas, Long Ashton Research Station, University of Bristol.

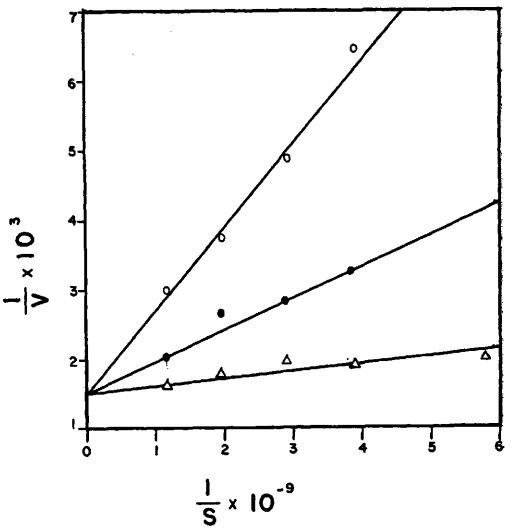

FIG. 1. Lineweaver-Burk plot showing competitive inhibition of molybdate by tungstate on growth of *A. niger* in a nitrate medium. V = mg dry wt of mycelium after 6 days. S = molar concentration of molybdate. △ = no tungstate. ● = 4.53 × 10^{-8} M tungstate. ○ = 9.06 × 10^{-8} M tungstate.

inhibition were obtained by adding tungstate at levels of 4.35 x 10^{-8} M and 9.06 x 10^{-8} M (0.5 and 1.0 μg W per 60 ml). Curves such as those shown in Fig. 1 were obtained and reproduced at various tungsten concentrations.

Molybdenum did not appear to be a necessary growth factor for this organism when ammonium ion was supplied as the nitrogen source (Table II). The basal medium was prepared by replacing the KNO_3 with an equivalent amount of NH_4Cl, and it was freed of trace amounts of molybdenum in the usual way(6). The ammonium medium not only supported better growth than the nitrate medium but the growth was unaffected by variations in Mo content. Growth on the

TABLE II. Effect of Mo and W on Growth of *Aspergillus niger* in Nitrate and Ammonium Media for 6 Days.

Metal content		mg dry mycelium	
mμg Mo	μg W	NO_3^- medium	NH_4^+ medium
0	0	140	993
0	2	—	1014
2.5	0	396	1010
2.5	2	29	1114
2.5	5	—	1111
1000	2	441	1088

nitrate medium containing 2.5 mμg of molybdenum was prevented by 2 μg of tungsten, but there was no decrease in growth with as much as 5 μg of W (the highest level tested) on the ammonium medium. Since growth was optimal on the ammonium medium and was unaffected by the Mo or W concentrations, the only apparent role for Mo in *A. niger* appears to be related to nitrate reduction.

Other ion relationships. Certain copper deficiency diseases in animals have been shown to be related to high dietary levels of molybdenum and inorganic sulfate(8,9). The converse effect of Cu or $SO_4^=$ on Mo deficiency was not observed with *A. niger*. On suboptimal levels of molybdenum (2.5×10^{-3} μg Mo) concentrations of copper or sulfate sulfur as high as 20 μg per 60 ml had no effect on growth. Manganous ion (40 μg/60 ml) also had no effect. Chromium (as K_2CrO_4) was studied in this system because of its association with Mo and W as a group VI_a element, but in molar Cr:Mo ratios as high as 15,000:1 chromium gave no growth inhibition. Bisulfite, tetraborate, chlorate, and ferricyanide were found to inhibit nitrate reduction by chicken liver xanthine dehydrogenase(10), a molybdoflavoprotein like nitrate reductase. However, when these substances were added to a nitrate growth medium containing 2.5×10^{-3} μg Mo in amounts which were equivalent on a molar basis to 2 μg W, no growth inhibition of *A. niger* resulted. Phosphotungstic acid was as effective a Mo antagonist as sodium tungstate, per equivalent of W.

Summary. When nitrate was the sole nitrogen source for *Aspergillus niger*, tungstate in a molar ratio of 20:1 with molybdenum readily produced growth inhibition, and a ratio of 400:1 prevented growth almost completely. The inhibition was entirely reversed by adding sufficient molybdate to give a W:Mo ratio of 1. The tungstate molybdate growth interaction yielded a competitive inhibition type of Lineweaver-Burk plot. The fungus displayed no requirement for molybdenum when grown on an otherwise adequate medium containing ammonium as the source of nitrogen.

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