various conditions have been given.

The results indicate that the alfalfa effect in the experiments of Cary and associates[4] is not due to the same factor for which our procedure tests. It appears likely that, aside from the principal factor under consideration the yeast and pure vitamin preparations which Cary and associates have in their diet do not satisfy the conditions for best growth of the young. Whatever significant nutritional factors occur in alfalfa are in our case adequately supplied by other diet components, probably the large amount of cottonseed flour.

Discussion. The data constitute evidence on two points: 1) that the amounts of alfalfa, dried grass or fresh grass fed do not contain effective amounts of zoopherin and 2) that whatever unknown factors affecting growth of young rats are contained in alfalfa are also otherwise provided by the all-plant ration. The first of these points stands on its own feet. The second becomes more significant if there is other evidence for a factor in alfalfa which can play the role we attribute to it in the experiments of Cary and associates.[4] Ball and Barnes[6] have reported that purified diets containing 8% of yeast are not adequate for lactation of mice (measured by the preweaning growth). Both dehydrated cereal grass and wheat bran effectively improved the situation but these two substances when added to the basal diet alone or combined did not give as good results as the stock commercial diet. Bowland, Ensminger and Cunha[7] in observations with purified diets (alcohol extracted casein, pure B factors, etc.) on rats have found that when 15% of dried young alfalfa was used as a supplement, 43% of the young given to the female to raise, were weaned. This contrasts with 13% weaned when the same 10 B factors, including folacin, were given without alfalfa. Spitzer and Phillips[8] have also shown evidence for a marked effect of alfalfa on lactation in rats. Their later brief communication,[9] however, indicates that at least a part of this is attributable to known factors.

Summary. A postweaning growth inhibition in the offspring of rats on a well fortified all-plant ration has been described. While this is corrected by a factor in liver, neither alfalfa leaf meal, dried grass nor young fresh grass have such an effect.

6 Ball, Z. B., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 692.

7 Bowland, J. P., Ensminger, M. E., and Cunha, T. J., *Arch. Biochem.*, 1948, **16**, 257.

8 Spitzer, R. R., and Phillips, P. H., *J. Nutrition*, 1946, **32**, 631.

9 Spitzer, R. R., and Phillips, P. H., *Fed. Proc.*, 1947, **6**, 422.

16508

Inhibition of Growth of the Vaccinia Virus by β-2-Thienylalanine and its Reversal by Phenylalanine.*†

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The effect of β-2-thienylalanine upon the growth of *Saccharomyces cerevisiae*, *Escherichia coli* and certain other microorganisms has been reported by du Vigneaud and coworkers.[1,2] Thienylalanine was observed to inhibit the growth of these organisms, and the inhibi-

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1 du Vigneaud, V., McKennis, H., Jr., Simmonds, Sofia, Dittmer, K., and Brown, G. B., *J. Biol. Chem.*, 1945, **159**, 385.

2 Dittmer, K., Ellis, G., McKennis, H., Jr., and du Vigneaud, V., *J. Biol. Chem.*, 1946, **164**, 761.

tion was counteracted by phenylalanine. These investigators found that as the concentration of phenylalanine in the medium was increased, larger amounts of thienylalanine were required to produce inhibition of growth.

In a previous report,[3] it was demonstrated that the growth of the vaccinia virus in cultures containing chick embryonic tissues was inhibited by a number of substituted amino acids. It was of interest, therefore, to determine whether thienylalanine might act in a similar manner. Experiments also were carried out to ascertain whether phenylalanine or methionine would neutralize the toxicity of thienylalanine for the virus.

Methods. The same strain of vaccinia virus was used for all experiments. Cultures were prepared in tubes with a diameter of 30 mm. The medium consisted of Tyrode's solution containing approximately 1% minced chick embryonic tissue and 2% normal rabbit serum. Supernatant fluid from a previously titrated culture served for the inoculum. The volume of fluid in each culture was 15 ml. Test materials were dissolved in distilled water. Solutions were adjusted to approximately pH 6.5 and filtered through Seitz filters.

Cultures were incubated at 33°C for 96 hours. The final concentration of virus in cultures was determined by the intradermal inoculation of 0.2 ml quantities of decimal dilutions in rabbits. The initial concentration of virus was calculated from data for the culture which supplied the inoculum. Titers were estimated by the method of Reed and Muench.[4]

Results. The results of experiments to determine the effect of thienylalanine, phenylalanine and methionine on the growth of the vaccinia virus are presented in Table I.

Phenylalanine in a concentration of 5×10^{-5} had no demonstrable effect on the multiplication of the virus. On the other hand, the addition of thienylalanine to cultures to give a final concentration of 5×10^{-4} produced a reduction in the concentration of virus. The addition of both compounds to

TABLE I.

Effect of β-2-thienylalanine, Phenylalanine, and Methionine on Growth of Vaccinia Virus in Chick Embryonic Tissues.

Metabolite	Analog	Concentration of: Metabolite	Concentration of: Analog	Change in titer of virus (log): In control cultures	In cultures containing: Metabolite	In cultures containing: Analog	In cultures containing: Metabolite and analog
dl-phenylalanine	β-2-thienylalanine	5×10^{-5}	5×10^{-4}	1.85	1.71	—1.02	1.19
,,	,,	5×10^{-5}	1×10^{-3}	0.90		—2.16	1.14
		2×10^{-5}	1×10^{-3}	,,			0.34
		1×10^{-5}	,,	,,			—0.07
Methionine	,,	1×10^{-4}	,,	1.80	0.62	—2.08	—1.98

3 Thompson, R. L., *J. Immunol.*, 1947, **55**, 345.

4 Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

cultures permitted viral multiplications.

In a second experiment a constant amount of thienylalanine was added to cultures while the concentration of phenylalanine was varied. The concentration of thienylalanine employed was 1×10^{-3} and the reduction in virus content of cultures was greater than that observed in the first experiment. A ratio of phenylalanine to thienylalanine of 1 to 20 permitted normal growth of the virus. With ratios of 1 to 50 and 1 to 100 a gradual decrease in the neutralization of the toxicity of thienylalanine for the virus was observed.

In view of the similarity in structure of thienylalanine and methionine, tests were made to determine whether the latter compound might counteract the toxicity of the analog for the vaccinia virus. A ratio of methionine to thienylalanine of 1 to 10 failed to permit viral multiplication.

Discussion. The use of metabolite antagonists has greatly added to our knowledge of the metabolism of bacterial cells.[5,6] It is reasonable to assume that these compounds likewise can supply valuable information concerning the nutritional requirements of viruses. The observation that thienylalanine inhibits multiplication of the vaccinia virus in chick embryonic tissues and that the toxicity of the analog is relieved by phenylalanine indicates that the metabolism of phenylalanine is in some way associated with the proliferation of the virus. Whether the action of thienylalanine is directed at the virus or at the parasitized cell or both remains to be determined. In any case, thienylalanine is an effective metabolite antagonist in the presence of living tissues.

5 Roblin, R. O., Jr., *Chem. Rev.*, 1946, **38**, 255.

6 Woolley, D. W., *Physiol. Rev.*, 1947, **27**, 308.

Summary. β-2-thienylalanine prevents the multiplication of the vaccinia virus in chick embryonic tissues. The toxicity is neutralized by phenylalanine but not by methionine.

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Growth of Neurotropic Viruses in Extraneural Tissues. I. MM Virus in the Feet of Hamsters.

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Evidence from many sources suggests that the viruses of poliomyelitis and mouse encephalomyelitis grow in extraneural tissues.[1] It is well known that these viruses occur frequently in the intestinal contents and feces of infected persons and laboratory animals. Numerous reports of finding them in extraneural tissues outside of the intestinal tract of infected persons and monkeys[2-18] and of rodents[19-26] have been published. There have been several reports of the growth of some strains in tissue culture[21,27,28] and in the chick embryo.[29,30,31] A precise identification of the extraneural tissues capable of supporting growth of these viruses in the intact animal has not been re-

1 Evans, C. A., and Green, R. G., *J. A. M. A.*, 1947, **134**, 1154.

2 Burnet, F. M., and Jackson, A. V., *Australian J. Exp. Biol. and Med. Sci.*, 1940, **18**, 361.

3 Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1919, **29**, 379.

4 Flexner, S., and Lewis, P. A., *J. A. M. A.*, 1910, **54**, 535.

5 Horstmann, Dorothy M., Melnick, J. L., and Wenner, Herbert A., *J. Clin. Invest.*, 1946, **25**, 270.

6 Horstmann, Dorothy M., Melnick, Joseph L., Ward, Robert, and Sá Fleitas, M. José, *J. Exp. Med.*, 1947, **86**, 309.