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.

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.

The authors are indebted to Dr. Karl Dittmer of the University of Colorado for the thienylalanine used in these experiments.

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

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

16509

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

C. A. EVANS AND VELMA C. CHAMBERS.

From the Department of Microbiology, School of Medicine, University of Washington.

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.

ported in the case of any one strain of virus or any one species of animal.

The relationship between the various strains of viruses designated as poliomyelitis and mouse encephalomyelitis is obscure. They clearly differ greatly in pathogenicity for different kinds of animals but there is at present no satisfactory means of classifying them. A knowledge of the ability of representative strains to grow in different kinds of tissue cells should be of value in developing a reasonable system of classification.

In the experiments reported in this paper, one member of this group, the MM virus, was shown to increase rapidly and extensively in the inoculated foot of the Syrian hamster.

The MM virus was originally isolated and described by Jungeblut and Dalldorf.[32] The virus used in these investigations was obtained from Dr. Gilbert Dalldorf in January 1944, and subsequently has been maintained by a total of less than 20 passages in mouse brains with intervals of storage as infected mouse brain in 50% neutral glycerin at 4°C. During the time the experiments were in progress it was shown to be noninfective for guinea pigs by subcutaneous inoculation and for rabbits by intra-ocular inoculation. Mice injected intracranially with the virus died at 2 or 3 days, or with minimal infective doses, after periods up to 7 days. Occasional mice exhibited extensive paralysis before death.

The hamsters used in these experiments were raised in the departmental animal colony and were 4 to 5 weeks old. Hamsters injected subcutaneously with MM virus regularly developed paralysis. There was no definite pattern to the areas of involvement, facial muscles, as well as limbs being paralyzed in various animals. A hamster inoculated in the right hind food after excision of a section of the right sciatic nerve developed extensive paralysis after the usual incubation period of 3 days.

Procedure. A stock supply of virus was prepared as a 20% suspension of infected mouse brain in 50% neutral glycerine. This was kept in a vaccine bottle at 4°C. Titrations showed that this stock suspension maintained its potency well throughout the period of the experiments.

Hamsters were injected subcutaneously in the pad of the right hind foot with 0.03 ml of

[7] Howe, H. A., Wenner, H. A., Bodian, D., and Maxcy, K. F., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 171.

[8] Howe, Howard A., and Bodian, David, *Am. J. Hygiene*, 1947, **45**, 219.

[9] Howe, Howard A., Bodian, David, and Wenner, Herbert A., *Bull. Johns Hopkins Hosp.*, 1945, **76**, 19.

[10] Kessel, J. F., Moore, F. J., Stimpert, F. D., and Fisk, R. T., *J. Exp. Med.*, 1941, **74**, 601.

[11] Kling, C., Olin, G., and Gard, S., *C. R. Soc. de Biol.*, 1938, **129**, 451.

[12] Melnick, Joseph L., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 14.

[13] Melnick, Joseph L., Horstmann, Dorothy M., and Ward, Robert, *J. Clin. Invest.*, 1946, **25**, 275.

[14] Paul, John R., Trask, James D., and Webster, Leslie T., *J. Exp. Med.*, 1935, **62**, 245.

[15] Sabin, A. B., and Ward, R., *J. Exp. Med.*, 1941, **73**, 771.

[16] Ward, R., and Waters, B., *Bull. Johns Hopkins Hosp.*, 1947, **80**, 98.

[17] Ward, R., Horstmann, D. M., and Melnick, J. L., *J. Clin. Invest.*, 1946, **25**, 284.

[18] Wenner, H. A., and Paul, J. R., *Am. J. Med. Sci.*, 1947, **213**, 9.

[19] Gard, S., *Yale J. Biol. and Med.*, 1944, **16**, 467.

[20] Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

[21] Jungeblut, C. W., Feiner, R. R., and Sanders, M., *J. Exp. Med.*, 1942, **76**, 31.

[22] Olitsky, P. K., *J. Exp. Med.*, 1940, **72**, 113.

[23] Sanz Ibanez, J., *Trab. d. Inst. Cajal de Invest. Biol.*, 1944, **36**, 137.

[24] Smith, M. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 88.

[25] Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, **72**, 49.

[26] Theiler, M., *Med.*, 1941, **20**, 443.

[27] Parker, Raymond C., and Hollender, Augusta J., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 88.

[28] Sanders, M., and Jungeblut, C. W., *J. Exp. Med.*, 1942, **75**, 631.

[29] Brutsaert, Paul, Jungeblut, Claus W., and Knox, Alice, *J. Pediatrics*, 1946, **29**, 350.

[30] Gard, S., *Nature*, 1943, **152**, 660.

[31] Schultz, Edwin W., and Enright, John B., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 8.

[32] Jungeblut, C. W., and Dalldorf, G., *Am. J. Pub. Health*, 1943, **33**, 169.

TABLE I.
Tests for Virus in the Blood and Central Nervous System of Hamsters Inoculated with MM Virus in the Hind Foot.
Exp. I. Sept. 1, '47.

Hr. after injection	12*	36*	61†
Dilution of tissue	10^{-1}	10^{-1}	10^{-1}
Blood	4	2	2
	S	3	3
	S	3	5
CNS	10	2	2
	S	3	2
	S	3	3

* Pool from 3 hamsters.
† Pool from 2 hamsters.
Hamsters in this experiment received 0.03 ml 10^{-3} dilution of MM virus.

Exp. II. Sept. 8, '47.

Hr after inj.	8	24			36			48		
Dilution of tissue	10^{-1}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
Blood*	4	3	2	3	3	2	3	2	2	2
	S	3	3	4	3	3	4	2	2	6
	S	3	3	S	3	4	6	2	2	S
CNS*	S	3	S	S	2	3	3	1	2	2
	S	4	S	S	3	3	3	2	2	2
	S	S	S	S	3	3	3	2	3	2

* Pool from 2 hamsters.
Hamsters in this experiment received 0.03 ml 10^{-4} dilution of MM virus.

inoculum. Subsequently various tissues were tested quantitatively for the amount of virus present.

Dilutions of virus, or of tissues being tested for virus, were prepared in Ringer's solution containing glucose. Tests for virus were all based on the intracranial injection of mice approximately 3 to 4 weeks of age.

Experimental. I. Determination of the approximate minimal infective dose (m.i.d.) for hamsters inoculated in the hind foot.

In a preliminary experiment it was determined that a 10^{-5} dilution of infected mouse brain (from the stock suspension) constituted approximately 1 m.i.d. of virus for hamsters inoculated in the foot pad. In subsequent experiments, hamsters were injected with a 10^{-4} dilution (10^{-3} dilution in one experiment) of infected brain from the same stock suspension.

II. Virus increases in the blood before it is detectable in the central nervous system (CNS).

In a series of several experiments (Table I) it was shown that after MM virus is inoculated subcutaneously into the pad of a hind foot of a hamster, a definite increase in the amount of virus in the blood precedes the appearance of and growth of virus in the central nervous system. In one experiment, for example, 28 hours after injection of virus into the foot pad, tests showed that 2 out of 3 mice injected with blood diluted to 10^{-2} were infected, but a 10% suspension of brain and spinal cord from the same hamster failed to infect mice.

The results strongly suggested that the virus was growing in the extraneural tissues. We next sought to determine the site of this growth of virus.

III. Virus increases in tissue at the site of injection before appearing in the blood.

Eight hamsters 34 days old from the same litter were inoculated in the right hind foot with a 10^{-4} dilution of infected mouse brain. Two animals were killed at 20, 24, 28 and 32 hours.

The initial injections were made at 9 A.M. (animals killed 24, 28, and 32 hours), and 1 P.M. (hamsters killed at 20 hours) on the

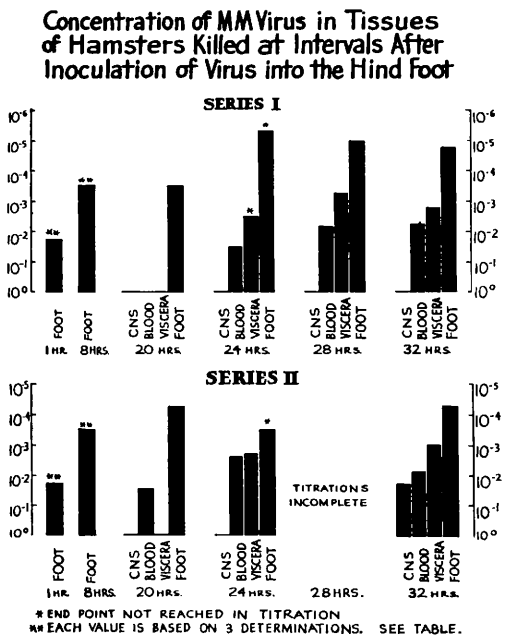

FIG. 1.
Concentration of MM Virus in Tissues of Hamsters Killed at Intervals After Inoculation of Virus into the Hind Foot.

Results of tests on individual hamsters killed at 20, 24, 28, and 32 hours are shown. Data for the 1- and 8-hour periods represent the average titers of 3 tests at each time period and are duplicated under Series I and Series II.

"Foot" designates tissue at the site of inoculation in the hind foot.

Note the early increase of virus in the foot with a subsequent rise in virus of the blood and viscera before it was present in the CNS.

same day. Titrations of virus in the inoculum showed that in spite of the fact that it was kept in the refrigerator from 9 A.M. to 5 P.M., a significant amount of virus died during this interval. Fortunately, the critical results were obtained with hamsters inoculated at 9 A.M.

Hamsters were killed with ether at the appropriate times on the following day. A sample of blood was collected from the heart. The brain and cervical spinal cord (CNS) were removed and tested for virus and the carcass placed in a freezer at –40°C.

At intervals of 3 to 19 weeks following the killing of the animals and the initial tests of blood and CNS, frozen carcasses were removed from the freezer and studied more extensively. There was no indication that appreciable amounts of virus died during storage. Tissues tested included local tissue from the site of injection of the foot, right popliteal lymph node, liver, kidney, spleen, and blood. The local tissue from the foot pad was obtained by reflecting the skin of the foot and dissecting off the remaining soft tissue of the plantar side of the foot. This was carefully weighed and amounted to from 16 to 31 mg. No inflammation was evident. The popliteal lymph nodes were found without difficulty and were determined to weigh from 2.2 to 7.0 mg. Portions of liver, spleen and kidney weighing approximately 0.1 to 0.5 g were tested individually or as pooled suspensions. In preparing the pooled suspensions, the portions of liver, spleen and kidney from the same animal were pooled in a sterile mortar, triturated thoroughly, and Ringer's solution added to make a 10% suspension.

Initial suspensions of abdominal viscera and CNS were 10^{-1}. In the case of local tissue and lymph nodes, 10^{-2} suspensions were the most concentrated that could be injected.

The results of this experiment are shown in Table II, and are presented graphically in Fig. 1. All end points were calculated by the method of Reed and Muench.[33] All deaths over 7 days were omitted in these calculations but are included in the tables.

It is clearly shown that within 32 hours after inoculation, virus attained a very high concentration in the tissues of the inoculated foot. In animals killed at the proper stage of the infection, there is a gradient of decreasing virus content from the foot (which contained the largest amount) to the regional lymph node, the blood and viscera, and central nervous system. In a single test the tissues of the uninoculated left hind foot of a hamster did not contain a detectable amount of virus at 32 hours, although there was sufficient virus in the inoculated right hind foot at this time to kill 3 out of 3 mice injected with a 10^{-4} dilution and 1 out of 3 injected with a 10^{-5} dilution of the tissue.

In subsequent experiments the inoculated

[33] Reed, L. J., and Muench, H., *Am. J. Hygiene*, 1938, **27**, 493.

TABLE II.
Quantitative Tests for MM Virus in Tissues of Hamsters After Injection of Virus into Right Hind Foot.

	Hamster I, 20 hr					Hamster I, 24 hr					Hamster I, 28 hr							Hamster I, 32 hr					
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
CNS	S	S				S	S				S	S						S	S				
	S	S				S	S				S	S						S	S				
	S	S				S	S				S	S						S	S				
Local tissue		3	3	S	S		2	2	3	4		2	2	3	4	S	10		2	2	3	4	S
		4	3	S	S		2	2	4	4		2	3	3	4	S	S		2	2	3	S	S
		4	7	S	S		2	3	4	S		4	3	10	S	S	S		2	3	4	S	S
Lymph node		3	S	S	S		2	2	4	3		2	1	S	10	S	S		2	2	S	S	
		5	S	S	S		2	2	4	4		2	3	S	S	S	S		2	3	S	S	
		S	S	S	S		2	2	4	S		3	3	S	S	S	S		3	S	S	S	
Blood	S	S				2	S				3,4*	3,S*	4*	S*				2,3*	3,2*	S*	S*		
	S	S				3	S				3,4	4,S	S	S				3,3	S,5	S	S		
	S	S				3	S				3,7	6,S	S	S				3,3	S,5	S	S		
Viscera	S	S	S	S	S	2	2				3	3	1	5	S			2	3	2	S	S	
	S	S	S	S	S	2	3				3	5	5	S	S			2	3	S	S	S	
	S	S	S	S	S	3	4				5	S	S	S	S			3	4	S	S	S	

Dilution	Hamster II, 20 hr					Hamster II, 24 hr			Hamster II, 28 hr			Hamster II, 32 hr					
	10-1	10-2	10-3	10-4	10-5	10-1	10-2	10-3	10-1	10-2	10-3	10-1	10-2	10-3	10-4	10-5	10-6
CNS	S	S				S	S		4	S		3	3				
	S	S				S	S		S	S		4	S				
	S	S				S	S		S	S		4	S				
Local tissue		2	2	3	S		2	2		2	2		2	2	3	S	S
		2	2	4	S		2	2		2	3		2	3	5	S	S
		2	3	S	S		2	3		3	3		2	3	S	S	S
Lymph node		S					2	2		2			2	1	3	S	S
		S					2	3		2			3	3	S	S	S
		S					2	3		3			4	3	S	S	S
Blood	3	4				2,3*	3,2*	5*	2	2		2,3*	3,3*	S*	S*		
	3	S				2,3	4,4	S	2	3		2,3	3,9	S	S		
	S	S				2,3	S,5	S	3	4		3,S	3,S	S	S		
Viscera	S											2	3	3	S	S	
	S											3	4	5	S	S	
	S											3	13	S	S	S	
						Tested separately			Tested separately								
Spleen						3	3	S	2	4	4						
						3	4	S	3	4	S						
						3	5	S	3	7	S						
Liver						3	3	3	3	2	4						
						3	3	4	3	4	7						
						3	4	S	3	4	S						
Kidneys						1	3	3	3	3	S						
						2	S	4	3	4	S						
						4	S	S	4	S	S						

CNS and blood of all hamsters were tested 9/16. Other tissues were tested on the following dates: Hamster I, 20 hr, 10/20; Hamster II, 20 hr, 1/26; Hamster 1, 24 hr, 11/3; Hamster II, 24 hr, 10/13; Hamster I, 28 hr, 11/29; Hamster II, 28 hr, 10/6; Hamster I, 32 hr, 11/17; Hamster II, 32 hr, 11/28.

A second test of blood of the following hamsters was made at the time other tissues were tested: Hamster II, 24 hr, Hamster I, 28 hr, Hamsters I and II, 32 hr.

TABLE III.
Tests for Amounts of Virus in Local Tissue of Hind Feet of Hamsters at Intervals of 1, 7, and 8 Hr After Injection of MM Virus.
Tests at 7 hr. (Exp. I. Injections Feb. 2, feet frozen over night before tests for virus.)

	Hamster I.		Hamster II.	
Dilution of tissue	10^{-2}	10^{-3}	10^{-2}	10^{-3}
	2	6	3	3
	3	S	3	6
	4	S	S	S

	Tests at 1 hr. (Exp. II. Injections Mar. 12).					
	Hamster I. Right hind foot		Hamster I. Left hind foot		Hamster II. Right hind foot	
Date of test*	Mar. 20, '48		Mar. 23, '48		Mar. 15, '48	
Dilution of tissue	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}
	3	S	3	S	4	S
	S	S	S	S	S	S
	S	S	S	S	S	S

	Tests at 8 hr. (Exp. II. Injections Mar. 12.)								
	Hamster II. Left hind foot			Hamster III. Right hind foot			Hamster III. Left hind foot		
Date of test*	Mar. 15, '48			Mar. 20, '48			Mar. 23, '48		
Dilution of tissue	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
	2	3	S	2	2	S	3	3	6
	2	4	S	3	3	S	3	4	S
	3	S	S	3	4	S	3	5	S

* Feet frozen until time of test.

feet of hamsters were tested at 1, 7 and 8 hours after injection of a 10^{-4} suspension of MM virus. The feet were frozen for 1 to 11 days before tests for virus were made. The results, as shown in Table III and in Fig. 1, indicate that even as early as 7 to 8 hours after injection there was considerable growth of virus in the inoculated feet.

Summary and conclusion. After inoculation into the pad of the hind foot of a hamster, MM virus increases in amount in the local tissue. Subsequent increases in virus in the blood and viscera are in turn followed by appearance of the virus in the central nervous system. It appears certain that the virus grows in the foot. Whether the growth occurs in subcutaneous tissue, muscles, or nerve endings was not determined in these experiments.

16510

The Mechanism of Egg-White Inhibition of Hemagglutination by Swine Influenza Virus.*

FRANK LANNI AND J. W. BEARD.
From the Department of Surgery, Duke University School of Medicine, Durham, N.C.

Egg-white (EW) has been found capable of inhibiting hemagglutination by purified preparations of swine influenza virus and derivatives obtained by heating the virus or treating it with formaldehyde.[1] This capacity

* This work was aided by a grant to Duke University from the Lederle Laboratories, Inc., Pearl River, N.Y.

[1] Lanni, F., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 312.