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Modification of Viral Synthesis in Tissue Culture by Substituting Pyruvate For Glucose in the Medium.* (22716)

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A previous publication(1) has shown that maximal propagation of the PR-8 strain of influenza virus by *in vitro* cultures of chorioallantoic membrane was dependent on readily available sources of energy. Recent studies revealed that penetration of the virus and utilization of cellular energy for viral synthesis were dependent also on adequate concentration of K^+, and that pyruvate provided a more sensitive system than glucose for studying relation of potassium deficiency to virus synthesis(2). It has now been observed that, while glucose as sole carbon source for cells permits them to propagate both the parent WS and the derived neurotropic NWS strains of influenza virus, the substitution of pyruvate for glucose as carbon source permits propagation of only the parent strain WS. It appears the differences between these two strains depends not solely on host cells chosen, but also upon subtle differences in relation between viral synthesis and sources of energy for the cells.

Materials and methods. Virus. Influenza A viruses, strains WS(3) and NWS(4), were maintained by propagation in the allantoic sac of 10- to 11-day-old chicken embryos. All viral inocula were from allantoic fluid stored in hermetically sealed vials in a dry ice chest. Dilutions were made in phosphate buffer pH 7.3 to 7.4. Egg infectivity titrations (EID_{50}) and reciprocal hemagglutinin titrations (HA) were conducted by usual methods(5,6). Tissue cultures were inoculated with 10^3 to 10^4 EID_{50}. All data represent average values of 3 experiments. The 112th allantoic sac passage of strain WS was received through the courtesy of Dr. R. R. Wagner. The 114th allantoic sac passage of strain WS was used in all experiments. Strain WS showed no neurotropic properties on intracerebral inoculation of mice. Lyophilized 8th mouse brain passage of strain NWS was kindly provided by Dr. R. W. Schlesinger. The 3rd allantoic sac passage of strain NWS was used in all experiments. In 7 subsequent intracerebral passages in mice strain NWS produced a uniformly fatal disease. *Tissue cultures and substrate.* Minced and washed chorioallantoic membranes from 10- to 12-day-old chicken embryos were used in all experiments, as previously described(2). Balanced salt solution (BSS)(7) without glucose and $NaHCO_3$ was used as the basal solution. Reagent grade† carbohydrates, pyruvate, etc. were used. The final medium was adjusted to pH 7.6 with sterile NaOH before addition of tissue and virus.

Results. Table I summarizes data showing that chorioallantoic membrane tissue cultures (CamTC) maintained in 0.1% glucose supported multiplication of influenza virus

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TABLE I. Multiplication of Strains WS and NWS in Tissue Cultures Provided with Pyruvate and with Glucose.

BSS plus:															
Pyruvate	0			.1%			0			0			.1 %		
Glucose	0			0			.1%			.02%			.02%		
	HA			HA			HA			HA			HA		
Virus (hr)	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
WS	<2	4	8	4	16	40	8	32	64	2	8	16	16	128	256
NWS	<2	<2	<2	<2	<2	<2	<2	32	64	<2	<2	±2	<2	128	128

BSS = Balanced salt solution without carbon source. HA = Reciprocal of avg viral hemagglutinin titers of 3 exp.

strains WS and NWS as measured by increases in HA, whereas in 0.1% pyruvate only strain WS multiplied. Cells in BSS without pyruvate or glucose supported multiplication of strain WS comparable with data obtained with strain PR-8 under similar conditions(1). CamTC in 0.02% glucose did not yield a significant increase in HA of strain NWS, however combination of 0.02% glucose and 0.1% pyruvate gave the highest HA observed for both viruses. In other experiments up to 5% pyruvate did not result in multiplication of strain NWS. In the presence of 0.1% pyruvate EID_{50} of tissue plus supernatant fluid determined at 12, 24, and 36 hours showed less than a 10-fold increase in infectivity of strain NWS, whereas increases of 1000-fold and greater were found with strain WS.

That the failure of CamTC to synthesize NWS virus, in presence of pyruvate, was not due to failure of virus to infect the cells, was shown in the following experiments similar to those previously reported in studies on potassium and infection with strain PR-8(2). Strain NWS at a titer of 10^3 EID_{50} was incubated with chorioallantoic membrane in the pyruvate medium for 2 hours and then anti-NWS rabbit serum was added. When the tissue was washed with physiological saline to remove the immune serum and fluids were replaced with 0.1% glucose medium, HA of 16 were detected after 72 hours. This result indicated that in the pyruvate medium strain NWS incubated with tissue became attached to cells (or penetrated cells) and was thus made insusceptible to neutralization by antibody.

The synergism observed with the combination of 0.02% glucose and 0.1% pyruvate suggests that glucose supplies factors essential for synthesis of strain NWS in CamTC. Compounds comparable to glycolytic products, Krebs cycle intermediates, high energy phosphates, coenzymes, etc. were tested for their influence on multiplication of strain NWS in CamTC. The data in Table II show that when CamTC was maintained in the presence of glucose, xylose, or glycerol, an increase in HA was observed. Ribose, dihydroxyacetone, glycerophosphate, adenosine-5

TABLE II. Effect of Various Substrates on Multiplication of Strain NWS in Tissue Cultures.

BSS + Substrate	.1% HA	.02% + .1% pyruvate HA
None	<2	
Na pyruvate	<2	
Glucose	32	128
D (+) Xylose	8	8
D (−) Ribose	<2	16
D (−) Arabinose	<2	<2
Dihydroxyacetone	<2	32
Glycerol	32	32
Na glycerophosphate	<2	8
NH_4 lactate	<2	<2
Na acetate	<2	<2
Adenine sulfate	<2	<2
Adenosine	<2	<2
" -5 phosphate	<2	32
" diphosphate	<2	32
" triphosphate	<2	32
Diphosphopyridine nucleotide	<2	64
Triphosphopyridine nucleotide	<2	4
Glutamine	<2	<2
Ca gluconate	<2	<2
Fumaric acid	<2	<2
Cis-aconitic acid	<2	<2
Isocitric acid	<2	<2
Malic acid	<2	<2
Succinic acid	<2	<2
Keto-glutaric acid	<2	<2
Oxalic acid	<2	<2

BSS = Balanced salt solution without carbon source.
HA = Reciprocal of avg viral hemagglutinin titers of 3 exp. after 48 hr.

phosphate, adenosine diphosphate, adenosine triphosphate, diphosphopyridine nucleotide, or triphosphopyridine nucleotide in combination with pyruvate resulted in an increase in HA, though they were without effect when tested individually as the sole carbon source. Results with arabinose, lactate, acetate, adenine, adenosine, glutamine, gluconate, fumaric acid, cis-aconitic acid, isocitric acid, malic acid, succinic acid, ketoglutaric acid, or oxalic acid were negative when tested as a sole carbon source and in combination with pyruvate.

Discussion. The data in Tables I and II suggest that propagation of strain NWS in cells of the chorioallantoic membrane may require nucleotides or carbon sources not supplied by cells in the pyruvate medium, which nevertheless support growth of the parent strain. The difference in requirements between strains NWS and WS may or may not be a variation associated with neurotropism. This can only be determined after the growth of other influenza strains on CamTC in pyruvate and glucose has been studied. It is believed that with CamTC in a pyruvate medium adsorption and penetration of strain NWS is possible but viral synthesis is markedly reduced. Diphosphopyridine nucleotide (coenzyme I) with pyruvate in CamTC had a greater effect on virus multiplication than triphosphopyridine nucleotide (coenzyme II) which might suggest greater specificity for coenzyme I. Possibly a coenzyme I linked reaction is indicated.

Summary. Chorioallantoic membrane tissue cultures (CamTC) in balanced salt solution (BSS) with pyruvate as the sole carbon source supported synthesis of influenza virus (strain WS) but not its neurotropic variant (strain NWS). Various other substrates were studied alone and together with pyruvate for their effect on synthesis of strain NWS in chorioallantoic membrane. Cells maintained in BSS containing glucose, xylose, or glycerol as the sole carbon source supported viral synthesis, while cells maintained in ribose, dihydroxyacetone, glycerophosphate, adenosine-5 phosphate, adenosine diphosphate, adenosine triphosphate, or diphosphopyridine nucleotide supported viral synthesis only in combination with pyruvate. It is suggested that synthesis of strain NWS in CamTC requires nutritional factors which are not supplied by cells maintained in a medium with pyruvate as the sole carbon source.

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Role of Mammary Glands on Weight of Suckling Rats. (22717)

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In nutritional studies there is a great need for employing rats showing uniformity in weight. In the weanling rats reared in these Laboratories it has not been possible to obtain appreciable uniformity in weights. The individual variation was considerable although the average weaning weight of animals born during different periods remained fairly constant.* It is now known(1) that the weaning weight of rat is influenced by the following factors: (a) the number of young the mother suckles, (b) the position of the litter in litter series, (c) the nature of the diet. While studying these aspects, it was noticed that

* Unpublished data.