end product. Only in the case of fucose with the milk polysaccharide was there indication of a more specific effect. *In vivo* only fucose exerted an inhibitory effect. This inhibition was limited to the milk polysaccharide and could not be demonstrated with hog gastric mucin or low molecular weight forms of bifidus factor. Neither did fucose have any effect on the growth of strains of *L. bifidus* which do not require the bifidus factor. The position of fucose in the milk polysaccharide appears to give it distinctive properties with respect to activity as bifidus factor.

Summary. 1. The effect of the constituent monosaccharides on the enzymatic inactivation of high molecular forms of bifidus factor has been studied. The most marked effect was observed with fucose when it was used with human milk or purified fractions from human milk. 2. The significance of this effect of fucose on the inactivation of the bifidus factor *in vitro* was emphasized by the experiments *in vivo*. L-Fucose markedly inhibited growth of *L. bifidus* var. *pennsylvanicus* when the source of bifidus factor was human milk, but not when it was supplied as mucin or the simple N-acetyl-D-glucosamine-containing compounds.

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Received July 13, 1956. P.S.E.B.M., 1956, v93.

Chemical Inhibitors of Theiler's Virus.* (22663)

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Certain amino acids, nucleoprotein derivatives and analogues as well as various other compounds, previously have been reported to inhibit propagation of Theiler's GD VII virus, *in vitro*(1-5). In the present study some additional chemicals have been tested. A preliminary report was given elsewhere(6).

Methods. Tissue cultures of minced brain tissue from newborn mice were made in 50 ml stoppered, Erlenmeyer flasks. Each flask contained 50-100 mg of tissue in 3 ml Simms' solution at pH 8-9 and approximately 100 intracerebral MLD_{50} of a tissue culture passage strain of Theiler's GD VII mouse encephalomyelitis virus. After incubation at 35-36°C for 2 days the pooled supernatant fluids obtained by centrifugation of the contents of 3 flasks were tested for viral content by hemagglutination of human red cells. Ordinarily titers of 1280-2560 are obtained by this procedure. The structure of the hydroxycytidine used as prepared in this laboratory has not been established with certainty. The Metuchen was kindly furnished by Dr. F. M. Berger, Wallace Laboratories, the thiosemicarbazone compounds by Dr. R. L. Thompson, Sterling-Winthrop Research Institute.

Results. Table I gives results of tests with various chemicals. Of the substituted pyrimidine nucleosides, the cytidine compounds are slightly more inhibitory than are the corresponding derivatives of uridine. Some of the naturally occurring deoxyribosyl nucleo-

* Aided by a grant from the National Institutes of Health.

TABLE I. Percentage Reduction of Hemagglutination Titers of Virus in Presence of Certain Compounds.

Compound	% reduction of titer	
Nucleosides	.5 mg/ml	.1 mg/ml
5-bromocytidine	98	0
5-chlorocytidine	95	0
5-hydroxycytidine	90	50
5-hydroxyuridine	75	0
deoxyadenosine	75	0
deoxycytidine	0	—
deoxyguanosine	75	50
	3 mg/ml	1 mg/ml
5-bromodeoxyuridine	—	0
5-hydroxydeoxyuridine	—	0
thymidine	0-50	0
deoxyadenosine	100	95
deoxycytidine	50	0
deoxyuridine	—	0
Pyrimidine derivatives		
deoxycytidine-5′-phosphate	—	0
2-amino-4 methylpyrimidine	100	0
4,6-diamino-2-thiopyrimidine	90	85
2,4-dichloropyrimidine	0	—
2,4-dichloro-6-methylpyrimidine	0	—
5-bromouracil	—	50
2-thiouracil	50	0
propylthiouracil	100	75
5-methyl-2-thiouracil	90	0
6-methyl-2-thiouracil	100	0
	.1 mg/ml	.01 mg/ml
Thiosemicarbazones		
butane-2,3-dione-2-methoxime 3-	85	50
2-thenaldehyde-	50	0
5-bromo-2-thenaldehyde-	—	0
2,3-butane oxime-	—	0
	.1 mg/ml	.05 mg/ml	.005 mg/ml
Other chemicals			
chlorpromazine	—	100	50
quinine dihydrochloride	100	—	0
bacitracin	90	50	—
polymyxin, penicillin, erythromycin	50	—	—
mycostatin	0-50	—	—

sides were inhibitory as were also some of the ribosyl nucleosides(3). The compounds listed under pyrimidine derivatives varied in their capacity to inhibit virus. Two of the 4 thiosemicarbazones tested were inhibitory. The more active of the 2 was previously found by others to be the most inhibitory for vaccinia virus(7). Of the other chemicals used, chlorpromazine and one not listed in Table I, Metuchen (2,3 - diethyl - 3 - propanediol dicarbamate), are known to have effects on the CNS. The latter compound was not active at a concentration of 0.1 mg/ml but did give 75% reduction in hemagglutination titer at a concentration of 1 mg/ml and complete inhibition at 3 mg/ml. Chlorpromazine was as inhibitory as any drug ever tested in our system. An *in vivo* trial of the drug was made in the following manner. Groups of 10 albino Swiss mice (10-15 g) were each given intraperitoneally (i.p.) 1 mg of chlorpromazine daily (2 mg daily is lethal). A control group of mice received saline instead of chlorpromazine. Seven hours after the first dose of drug and immediately prior to the second dose, each mouse was given an intracerebral (i.c.) injection of 0.03 ml of tissue culture virus (approx. 1-10 MLD_{50}). The test mice died 3 to 8 days after virus injection as follows 3,3,3,3,7,7,7,7,8: deaths of control mice were at 4,7,7,7,8,9,10 days and 2 survived. In a second experiment drug treatment was given as above daily beginning on the third day after virus inoculation. Mice died on the days indicated after virus injections; test mice, 5,5,6,7,7,10,11, 3 survivors; control mice, 4,6,7,7,10,10,10, 2 survivors. The drug did not protect mice from viral death. The early deaths in the first experiment possibly resulted from a combination of viral effect and drug toxicity.

Bacitracin polymyxin, penicillin and erythromycin were found to inhibit virus; streptomycin, tetracycline, oxytetracycline and chloramphenicol did not inhibit. Mycostatin irregularly gave inhibition; possibly this irregularity is associated with the poor solubility of the drug. Polymyxin was used to treat mice which had received 1 MLD_{50} of virus i.c. 3 days previously. The mice were given 0.2 mg i.p. daily; control mice received saline. Control and test mice died 5-9 days after virus injection. The drug did not affect the infection in mice. Under the conditions of this experiment 0.3 mg daily of polymyxin was a fatal dose.

Inhibition of virus by 5-hydroxyuridine was reversed by uridine-5′-phosphate as shown in Table II. Comparable reversal of this inhibition also occurred with uridine(3).

Glutamine has been found by others(8) to be necessary for the propagation of poliovirus

in HeLa cell tissue cultures. This compound inhibited GD VII virus in our tissue culture system. The hemagglutination titer was reduced 75% by a concentration of 0.1 mg/ml.

Discussion. Uridine does not inhibit virus and partially reverses inhibition produced by some substituted uridine nucleosides(3). As shown above uridine-5'-phosphate also reverses the inhibitory effect of the nucleoside derivative. 5-hydroxyuridine. The greater activity of cytidine derivatives in comparison with uridine derivatives as shown here is similar to the findings for *Neurospora*(9). Although chlorpromazine was active, *in vitro*, it failed to influence the infection in mice. The quantity used in the tests was nearly the maximal amount tolerated since it was found in preliminary studies that 2 mg daily was a lethal dose. The dose of 1 mg used resulted in generalized muscular weakness from which recovery was gradual and not complete as long as 24 hours later. The drugs tested probably did not act by interfering directly with the hemagglutination test since control tests with representative drugs from each group and virus incubated for 1 hour at room temperature gave the same hemagglutination titer as virus alone with one partial exception; the RBC sediment pattern in the presence of 0.1 mg/ml quinine dihydrochloride did not have as definite an endpoint as the control tube with virus.

Summary. Certain pyrimidine-related-compounds inhibit Theiler's virus, *in vitro*. Inhibition by 5-hydroxyuridine is partially reversed by uridine-5'-phosphate. Of various other chemicals found to inhibit virus, *in vitro*, chlorpromazine and polymyxin did not protect mice from viral infection.

TABLE II. Reversal of 5-Hydroxyuridine Inhibition by Uridine-5'-phosphate.

	% reduction of hemagglutination titer
5-hydroxyuridine, .5 mg/ml	75
Uridine-5'-phosphate, .5 mg/ml	0
5-hydroxyuridine, .5 mg/ml + uridine-5'-phosphate, .5 mg/ml	50
5-hydroxyuridine, .5 mg/ml + uridine-5'-phosphate, 1 mg/ml	0

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Received July 13, 1956. P.S.E.B.M., 1956, v93.

Relation of Endocrines and Clearing Factor Inhibitors to Hyperlipemia in Fasted Animals.*† (22664)

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A variety of substances inhibit the clearing action of lipoprotein lipase *in vitro*. These include protamine(1), polylysine(2), diisopropylfluorophosphate (DFP)(3), iodinated thyronines(4), Na cholate(5), and an inhibitor (LM) present in plasma of rats receiving cortisone(6). Of these protamine(7), polylysine (2), Na cholate(5), and LM(8) have been reported to produce hyperlipemia when injected

* Presented in part at Fed. Am. Soc. Exp. Biol., April 16-20, 1956, Atlantic City, N. J.

† We wish to acknowledge the technical assistance of Wm. H. Bechmann, J. Dachiw, J. R. Howitz, M. R. Klenk, E. E. Livingston, Jr. and R. Reifsnyder.