quence(12,13,14). Initially, within the first 10 days following castration, there is a lowering of gonadotrophic potency which is predominantly FSH with little or no LH (ICSH). Twenty-one days post-castration, the pituitaries become increasingly rich in LH (ICSH). Our studies have shown that TEM, X-ray(5), microwaves(10) and surgical cryptorchidism(15), experimental conditions which do not injure Leydig cells morphologically or functionally, but which do damage seminiferous epithelium, also produce a temporary decrease in ICSH output followed by an increase similar in time sequence to that following castration. These studies furnish more evidence for the concept that the state of seminiferous epithelium is an important regulator of pituitary function.

Summary. Five to six days following the termination of a 5-day treatment of rats with TEM (0.2 mg/kg daily), the weight and capacity of the dorsolateral prostate to take up Zn^{65} was diminished, indicative of a diminished androgen level. Dorsolateral prostate weight and Zn^{65} uptake were maintained in TEM-treated rats by administration of testosterone, pituitary ICSH or chorionic gonadotrophin.

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Inhibitory Effect of Antiviral Compounds on Viruses *in vivo* and in Mouse Ascites Cells *in vitro*.* (28120)

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Antiviral effects of several compounds, such as hyroxybenzylbenzimidazole, 5-iodo-2′-deoxyuridine, fluorophenylalanine, N-methylisatin-thiosemicarbazone, ammonium salts, Statolon, and Helenine have been demonstrated by a number of workers(1-19). We have compared the effects of several such compounds against adapted strains of Columbia SK and LCM viruses propagated in mouse ascites tumor cells. Effects of certain of the compounds *in vivo* are also included.

a. In vitro *tests. Materials and methods.* 1. *Virus-host system.* Hemagglutination(HA)-positive and HA-negative strains of Columbia SK (Col SK) virus, and LCM virus, strain NY621, isolated from a leukemic L_2C

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TABLE I. Compounds tested.

Abbreviation	Source*	Name	Susceptible virus
6270-A	a	10-(3-dimethylamino-2-methyl-propyl)-2-methyl-thiophenothiazine-HCl	influenza PR8(2)
8706-C	a	SK&F derivative A	Rous saracoma(2)
11381-C	a	SK&F derivative B	mouse hepatitis(2)
5-IDU	a, b	5-iodo-2′-deoxyuridine	herpes simplex(2) vaccinia(2 to 5) Columbia SK(2)
HBB	a, c	2-(α-hydroxybenzyl)-benzimidazole	enteroviruses(1,2)
CH_3-HBB	a		parainfluenza-3(2)
CF_3-HBB	a		ECHO-9, Col SK(2)
HBB-Cl	a		" " (2)
Statolon	d	L209-617B-103-4 and L209-617B-111-4	polio(15) Rous sarcoma(16) Coxsackie A21(17)
Helenine	d	L571-848-06 and L571-848-07	swine influenza(18) Columbia SK(18) polio(19)
Methyl-ITSC	b	N-methylisatin-thiosemicarbazone	vaccinia(11,12)
FPA	e	DL-p-fluorophenylalanine	adeno(7) polio(8,9) fowl plaque(19)
Ammon. sulf.	e	ammonium sulfate	influenza PR8(13) Columbia SK(14)
PTA	e	phosphotungstic acid	
Methyl-GAG	f	methylglyoxal-bis-guanylhydrazone	anticancer(24)
5375-A	g	1,6-dinitronaphthalene	
5375-B	g	1,7- "	
5378	g	dihexachlorocyclopentadiene adduct of naphthalene-3-sulfonic acid-2-carboxylic acid	
3875	h	phenazine-di-N-oxide	anticancer(25)
B12	i	vit B_{12}	

* a. Smith Kline & French Labs., Philadelphia, Pa.
b. Dr. Randall L. Thompson, Nat. Cancer Inst.
c. Merck Sharp & Dohme Res. Lab., Rahway, N. J.
d. Lilly Res. Lab., Indianapolis, Ind.
e. Commercial.
f. Dr. F. French, Mt. Zion Hosp., San Francisco, Calif.
g. Dr. Arthur Furst, Inst. of Chem. Biol., Univ. of San Francisco.
i. Bioferm. Co., Wasco, Calif.

guinea pig by Dr. Claus W. Jungeblut of Columbia University(20), which have been adapted to Ehrlich ascites tumor cells in this laboratory were used in such cells(21,22). After washing, 2 to 4 $\times$ 10^6 Ehrlich ascites cells freshly harvested from mice were suspended in 1 ml of culture medium per tube. The medium was composed of 85% of Medium 199 and 15% of heat-inactivated (60°C, 30 min) Ehrlich ascites fluid from which fibrin had been removed by standing at 4°C for a week. Virus was added as 0.05 ml of a definite dilution of virus in Medium 199; compounds were added as 0.05 ml of aqueous solutions or suspensions just before virus inoculation. The same volumes of Medium 199 and of distilled water were added to controls. The stationary cultures were incubated at 36°C. After 3 or 4 days incubation, cultures were shaken by hand to remove the cells from the glass wall, and then centrifuged. The supernatant was used for titration of infectivity and HA; the cells were resuspended in saline and stained with 1 drop of 0.4% of erythrosin which colors the damaged cells red but does not stain living cells. With this staining method, the cytopathic effect (CPE) of the virus and the toxicity of the compounds could be detected quantitatively with a counting error within 10%. More than 75% loss of live cells, as compared with the controls, was recorded as 4+ virus growth; 74% to 50% as 3+; 49% to 25% as 2+; under 24% as 1+. For HA, a 0.2% suspension of sheep red cells in potassium barbital buffer was used. The infectivity ($TCID_{50}$) was determined in culture tubes as previously described(21,22). 2.

TABLE II. Inhibitory Effect of Compounds Against Cytopathic Effect, Hemagglutinin and Virus Production of Columbia SK Virus (HA-positive).

		Concentration of compound per ml of medium								
		1:2		1:4			1:8		1:16	
Compound	Minimum toxic dose	CPE	HA	CPE	HA	$TCID_{50}$	CPE	HA	CPE	HA
6270-A	25 μg	4+	1280	4+	1280		4+	1280		
8706-C	50	1+	40	3+	320	6.5*	4+	1280	4+	1280
11381-C	80	4+	1280	4+	1280		4+	1280		
5-IDU	500	3+	320	4+	1280	6.8	4+	1280		
HBB	1000	0	<10	0	<10	3.5	1+	40	4+	640
CH_3-HBB	125	4+	1280	4+	1280		4+	1280		
CF_3-HBB	125	3+	320	4+	1280		4+	1280		
HBB-Cl	32	4+	640	4+	1280		4+	1280		
Statolon-103	5000	0	<10	0	20	4.5	1+	640	4+	1280
Statolon-111	5000	3+	640	4+	1280	6.5	4+	1280		
Helenine-06	6000	4+	1280	4+	1280		4+	1280		
Helenine-07	5600	4+	1280	4+	1280		4+	1280		
Methyl-ITSC	300	4+	1280	4+	1280	6.5	4+	1280		
FPA	500	0	<10	0	<10	3.5	2+	320	4+	1280
Ammon. sulf.	5000	0	<10	0	<10	2.8	1+	320	4+	1280
PTA	1000	0	<10	0	20	4.5	2+	320	4+	1280
Methyl-GAG	500	0	<10	0	<10	3.2	0	10	3+	320
5375-A	1000	1+	20	2+	80	5.5	4+	1280	4+	1280
5375-B	150	4+	1280	4+	1280		4+	1280		
3875	20	4+	1280	4+	1280		4+	1280		
5378	350	0	<10	0	<10	4.5	0	20	3+	640
B12	400	0	<10	0	<10	3.5	0	10	3+	320
Control (H_2O)	0.2 ml	4+	1280	4+	1280	7.5				

* Indicates $10^{6.5}$ per 0.1 ml of harvest 3 days after inoculation of 1000 $TCID_{50}$ of virus.

Compounds tested. Table I lists the compounds tested, their source, and the viruses previously reported to be susceptible.

Results. 1. *Inhibitory effect of compounds against HA-positive Col SK virus.* Over 25 experiments were done with HA-positive Col SK virus from the 6th up to 19th passage (T6 to T19; these generations consistently produce high titers of HA). Each compound was tested at least 3 times. Throughout the experiments, inoculum size was about 1000 $TCID_{50}$, the compounds were added just before inoculation, and incubation time was 3 days. Table II shows the results. It was found that HBB, Statolon (L209-617B-103-4), FPA, ammon. sulf., PTA, methyl-GAG, 5375-A, 5378, and B12 could suppress virus multiplication with one-fourth of the minimum toxic dose, while another sample of Statolon, 5-IDU, methyl-HBB, CF_3-HBB, HBB-Cl, Helenine, 5375-B (an isomer of 5375-A), 3875 (an antineoplastic agent), and methyl-ITSC were not effective.

2. *Inhibitory effect of compounds against HA-negative Col SK virus.* Nine compounds effective against HA-positive virulent Col SK virus and 6 ineffective compounds were selected to test for activity against HA-negative avirulent Col SK virus, which was derived from HA-positive virus after many passages in Sarcoma 180 ascites cells *in vitro* (23). At present, the virus can propagate with 3+ CPE in Ehrlich ascites cells *in vitro*. Generations 60 to 80 of virus were used for these experiments. Each compound was tested at least 3 times in 8 experiments. The inoculum size was about 1000 $TCID_{50}$, the compounds were used at one-fourth minimum toxic doses, and incubation time was 4 days. Table III shows the results. Among the compounds, only 5378, PTA, Statolon (L209-617B-103-4) and B12 were effective. Unexpectedly, HBB, FPA, ammon. sulf., methyl-GAG and 5375-A which were effective against HA-positive Col SK virus were found to be ineffective against HA-negative virus. This dissociation had been previously reported with ammon. sulf.(14).

3. *Inhibitory effect of compounds against LCM virus.* The LCM virus was adapted to propagate in Ehrlich ascites cells *in vitro* in this laboratory and produced 3+ CPE. Fifteen compounds which were used against HA-negative Col SK virus were tested against the

TABLE III. Inhibitory Effect of Compounds Against Cytopathic Effect and Virus Production of Columbia SK Virus (HA-negative).

Compound	Dose*	CPE†	$TCID_{50}$†
8706-C	13 μg	3+	
5-IDU	125	3+	6.5
HBB	250	3+	6.5
CF_3-HBB	31	3+	
Statolon-103	1250	0	4.5
Statolon-111	1250	3+	6.5
Helenine-06	1500	3+	6.5
Methyl-ITSC	75	3+	6.5
FPA	125	3+	7.5
Ammon. sulf.	1250	3+	6.5
PTA	250	0	3.8
Methyl-GAG	125	3+	6.5
5375-A	250	3+	
5378	88	0	4.5
B12	100	0	3.5
Control (H_2O)	.05 ml	3+	7.2

* ¼ of minimum toxic dose.
† 4 days after inoculation of 1000 $TCID_{50}$ of virus.

LCM virus. Table IV shows the results of 10 experiments. HBB, methyl-GAG, and 5378 were effectively antiviral, but the activity was limited to CPE, and did not effect virus multiplication. Only B12 was found to be effective against both CPE and multiplication.

b. In vivo *test. Effect in mice.* A number of the compounds were tested for antiviral effect in mice. Administration was subcutaneous or oral, and was started at the time of virus inoculation, or 2 days before, in the general manner previously described(26). Infection was with Columbia SK or ectromelia virus. The results are shown in Table VI. It will be noted that outstanding antiviral effects are much less frequent than with *in vitro* testing. Only Statolon gave strikingly effective results, and this only in Columbia SK virus infection, and when given 2 days before virus inoculation. Methyl-ITCS was suggestive, also against Columbia SK virus.

Discussion. A number of compounds were tested for *in vitro* antiviral effects against 3 different viruses (Table V), with the demonstration of quite different ranges of effects. It was found that the antiviral activity of a compound might differ even between 2 strains of Col SK virus having the same antigen(22). The compounds were selected because of previously noted antiviral or anticancer effects, as cited in the references, except as follows; PTA was included as a control protein precipitant for ammon. sulf.; B12 because of its presence in crude effluents containing propionin(23); the 2 nitrophthalenes because of our observation of marginal effects with a number of aromatic nitro compounds. It is of interest that LCM virus could propagate freely in the presence of several compounds despite inhibition of CPE. It should be noticed that vit. B12 could suppress both the CPE and the multiplication of

TABLE IV. Inhibitory Effect of Compounds Against Cytopathic Effect and Virus Production of LCM Virus.

	Dilutions from minimum toxic dose						
	1:2		1:4		1:8	1:16	1:32
Compound	CPE*	$TCID_{50}$*	CPE	$TCID_{50}$	CPE	CPE	CPE
8706-C	3+	6.8	3+	7.5	3+	3+	
5-IDU	3+		3+		3+	3+	
HBB	0	6.5	0	6.5	0	2+	3+
CF_3-HBB	3+		3+		3+	3+	
Statolon-103	3+	6.5	3+	6.8	3+	3+	
Statolon-111	3+		3+				
Helenine-06	3+	6.5	3+				
Methyl-ITSC	3+	6.5	3+	6.5	3+	3+	
FPA	3+	6.8	3+	6.5	3+	3+	
Ammon. sulf.	3+	6.8	3+	7.5	3+	3+	
PTA	3+	6.5	3+		3+		
Methyl-GAG	0	6.5	0	7.2	0	2+	3+
5375-A	3+		3+		3+		
5378	0	6.5	0	6.5	1+	3+	
B12	0	3.5	0	3.5	0	2+	3+
Control (H_2O)	3+	6.5 to 7.5					

* 4 days after inoculation of 1000 $TCID_{50}$ of virus.

TABLE VI. Comparison of Effects of Antiviral Compounds Against Columbia SK and Ectromelia Infections in Mice.

	Columbia SK					Ectromelia				
				Deaths§					Deaths§	
Compound	Dose*	Days†	Start‡	C	T	Dose	Days	Start	C	T
6270-A	.02 mg	4	0	29/40	8/10	.02 mg	4	0	20/20	7/10
8706-C	3.0	4	0	29/40	8/10	3.0	4	0	20/20	7/10
11381-C	1.5	4	0	29/40	8/10	1.5	4	0	20/20	7/10
5-IDU	3.0	4	0	29/40	8/10	3.0	4	0	20/20	7/10
						2.5	7	0	14/20	8/10
						.2% diet	14	0	14/20	6/10
HBB	4.0	4	0	29/40	8/10	4.0 mg	4	0	20/20	5/10
						.1% diet	14	0	17/20	8/10
CH_3-HBB	4.0	4	0	29/40	7/10	4.0 mg	4	0	20/20	5/10
						.1% diet	14	0	17/20	9/10
CF_3-HBB	8.0	4	0	29/40	4/10	8.0 mg	4	0	20/20	5/10
	.1% diet	9	0	31/40	6/10					
HBB-Cl	8.0 mg	4	0	29/40	5/10	8.0	4	0	20/20	8/10
Statolon-103	6.0	4	0	34/40	9/10					
Statolon-111	6.0	6	−2	20/20	5/40					
	4.0	6	−2	33/40	13/40					
Methyl-ITCS	2.5 mg	6	−2	53/60	39/60	2.5 mg	7	0	14/20	7/10
	.2% diet	9	−2	20/20	13/20	.2% diet	14	0	14/20	6/10
Ammon. sulf.	.7 mg	4	0	69/80	36/40					
FPA	.5	4	0	56/80	23/40					
Methyl-GAG	.5	4	0	25/40	30/40	.05% diet	14	0	16/20	16/20
						.2	14	0	28/40	8/10
5375-A						.1	14	0	17/20	8/10
5375-B						.1	14	0	17/20	10/10
5378	1.0	4	0	34/40	9/10	.1	14	0	48/60	34/50
						.2	14	0	68/80	52/70
						.4	14	0	52/60	33/40
3875	1.5	5	0	17/20	19/20	1.5 mg	5	0	55/70	32/50
	.05% diet	4	0	7/10	9/10	.1% diet	14	0	13/20	14/20
B12	.01 mg	4	0	14/20	18/20					
	.05	4	0	36/40	33/40					
	.1	4	0	14/20	18/20					

* Given subcutaneously twice daily unless indicated as given in diet.
No. of days treated.
Day treatment started: 0 = day of virus inoculation; −2 = two days before virus.
C = control; T = treated.

TABLE V. Comparison of Inhibitory Effects of Antiviral Compounds on 3 Viruses.

	Columbia SK (HA-positive)		Columbia SK (HA-negative)		LCM (NY#621)	
Compound	CPE	$TCID_{50}$	CPE	$TCID_{50}$	CPE	$TCID_{50}$
8706-C	±*	—	—	—	—	—
5-IDU	±	—	—	—	—	—
HBB	+	+	—	—	+	—
CF_3-HBB	+		—	—	—	
Statolon-103	+	+	+	+	—	—
Statolon-111	—	—	—	—	—	
Helenine-06	—		—	—	—	
Methyl-ITSC	—	—	—	—	—	—
FPA	+	+	—	—	—	—
Ammon. sulf.	+	+	—	—	—	—
PTA	+	+	+	+	—	—
Methyl-GAG	+	+	—	—	+	—
5378	+	+	+	+	+	—
B12	+	+	+	+	+	+

* + = effective; ± = doubtful; — = not effective.

the LCM virus and Col SK virus in Ehrlich ascites cells *in vitro*. Eggers and Tamm(1) reported that several enteroviruses (RNA viruses) were susceptible to HBB, while Herrmann(4) showed that vaccinia and herpes simplex viruses (DNA) were susceptible to 5-IDU, but West Nile and Newcastle disease viruses (RNA) were not susceptible. The susceptibility of Col SK (HA-positive) virus to HBB, and its resistance to 5-IDU are in accord with the general assumption that it is an RNA virus. In contrast to the *in vitro* effectiveness of these compounds, only Statolon was clearly effective against Columbia SK virus in the mouse, and none was of outstanding effectiveness against ectromelia virus.

Summary. The antiviral activities of a number of known antiviral compounds, including HBB, 5-iodo-2′-deoxyuridine, fluorophenylalanine, N-methylisatin-thiosemicarbazone, ammonium sulfate, Statolon and Helenine on Columbia SK and LCM virus propagation in cultures of mouse ascites tumor cells have been described. These compounds were also compared with several other compounds not previously recognized as antiviral: methylglyoxal-bis-guanylhydrazone, 1,6-dinitronaphthalene, phosphotungstic acid, the dihexachlorocyclopentadiene adduct of naphthalene-3-sulfonic acid-2-carboxylic acid and vit. B12. Statolon was effective against Columbia SK infection in the mouse when given prophylactically 2 days before inoculation of the virus.

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