pirates of human γ and β_2A myelomas were smeared and labelled by fluorescent antibodies against γ and β_2A globulin. Never were all the observed plasma cells reactive with fluorescent antibodies, and in half of the cases, no fixation by any plasma cell was noted. Plasma cells of different types were fluorescent, most of them having a granular structure. They were found to fix fluorescent antibodies against γ globulin or β_2A globulin, but never both.

1. Burtin, P., *Rev. Fse. Et. Clin. Biol.,* 1961, v6, 1044.

2. Golstein, G., Slyzys, I. S., Chase, M. W., *J. Exp. Med.,* 1961, v114, 89.

3. Solomon, A., Fahey, J. L., Malmgren, R. A., *Blood,* 1963, v21, 403.

4. Vazquez, J. S., *J. Lab. Clin. Med.,* 1958, v51, 271.

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"Cyclopin": A Trypsin Sensitive Constituent of *Penicillium cyclopium* With Antiviral Properties.* (28618)

KIARASH NAFICY AND DAVID H. CARVER (Introduced by J. F. Enders)

Research Division of Infectious Diseases, Children's Hospital Medical Center, Department of Pediatrics, Harvard Medical School, and Children's Hospital Medical Center, Boston, Mass.

During an experiment with Sindbis virus in human amnion cell cultures, it was noted that in one culture the expected cytopathic changes did not occur. It was then observed that a fungus was growing in this tube. The fungus was cultured in Sabouraud's medium and identified as a penicillium by Dr. George Foley and Professor William H. Weston of Harvard University and subsequently as *Penicillium cyclopium* by Dr. Kenneth Raper of University of Wisconsin.

Preliminary tests revealed that cytopathic effects of Sindbis virus were regularly suppressed by extracts prepared from the penicillium. Experiments described below were then performed in attempts to elucidate the mechanism involved in inhibition of Sindbis virus by the extracts and to define some of the physical and chemical properties of the inhibitor. Information was also sought regarding the spectrum of viruses inhibited by this factor (or factors) in the fungal extract which for convenience of reference is here provisionally designated "cyclopin."

Materials and methods. Cell cultures. Primary cultures of human amnion (HA) and chick embryo (CE) cells were prepared as previously described(1,2). Human amnion cells were kept for at least 3 weeks prior to inoculation of arboviruses(3). Grivet monkey kidney *(Cercopithecus aethiops)* (MK) cell cultures were obtained commercially.†

Maintenance medium. Bovine amniotic fluid (BAF) was used in all cultures(4).

Viruses. The following viruses were employed:

Sindbis: Strain AR339 Taylor, A. R. CE 10, HA 4.

Western Equine Encephalitis (WEE) Johnson, H. Bird 628, Tc 00218, P15, cl 1 HA8.

West Nile: Sarafend Strain, Appendix cell line 6, CE 8.

Chickungunya: Suckling Mouse Brain 3, HA 2.

ECHO 11: Gregory Strain, MK 6, HA 4.

Poliovirus Type I: Brunhilde. Stock strain.

Herpes simplex: Zacchardi Strain, rabbit cornea 1, chick amniotic sac 5, CE3, HA 6.

Adenovirus Type II: Laboratory Strain Norian, WS 2 (stable line of HA) HA 4.

Coxsackie A9: Laboratory Strain Berkman, MK 4.

Coxsackie B2: Laboratory Strain Kransler, MK 6.

Coxsackie B5: Laboratory Strain Keating, MK 5.

Antisera. Sindbis rabbit antisera were pre-

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† Microbiological Associates, Bethesda, Md.

pared and kindly supplied by Dr. Elmer Pfefferkorn.

Infectivity assays. Extra-cellular virus (ECV) was obtained in the form of medium from infected tissue cultures. It was centrifuged at 2000 rpm for 10 minutes and serial 10-fold dilutions of supernatant fluid were prepared in BAF medium. Three cultures were inoculated with 0.1 ml of each dilution tested.

Intra-cellular virus (ICV): Infected tissue cultures were washed 5 times with 5 ml of medium 199, and the fifth washing was tested for viral infectivity in CE cells. All tests were negative. Cells were scraped from the culture tubes, mixed with 1 ml of BAF, frozen and thawed 3 times, and handled in the same manner as ECV.

Viral titers were calculated according to the method of Reed and Muench(5) and are expressed as log TCD_{50}/0.1 ml of fluid.

Scale of cytopathic effect. Viral cytopathic effects were scored as follows:

Estimated Percentage of Cells Affected:

0?	=	Equivocal changes in scattered foci of cells.
±	=	10
+	=	10-25
++	=	25-50
+++	=	50-75
++++	=	75-100

Enzymes. RNA-ase crystalline salt free‡, DNA-ase crystalline§, and 2X salt free Trypsin§ were obtained commercially.

Hemagglutin assay of arboviruses. This was carried out by the procedure of Clarke and Casals(6).

Growth of the fungus. The penicillium grew well at room temperature in various liquid media or Sabouraud's agar. In liquid medium when continuously shaken luxuriant growth occurred in fluffy, round, white masses. By the 3rd or 4th day each mass was approximately 3-6 mm in diameter. Growth was scanty and slow at 37°C; on occasion no growth at this temperature was noted.

Preparation of cyclopin. Fungus grown in Sabouraud's broth was harvested on the third or fourth day. Approximately 0.5 to 1 g was suspended in 25 ml of BAF, saline or distilled water, and subjected to sonic vibration for a period (usually 35-45 minutes) sufficient to disperse the masses completely and give a milky appearance to the preparation. If the pH appeared to be low, enough 2.4% sodium bicarbonate was added to raise the pH to 7-8. The preparation was then centrifuged at 5000 rpm for one hour, and supernatant fluid was passed through a Millipore filter (pore size 0.45 micron), and assayed.

Assay of cyclopin and definition of activity unit. Serial 2-fold dilutions of fungal extract were prepared in BAF. Aliquots of 1 ml of each dilution were introduced into each of 2-3 human amnion cultures that were immediately inoculated with 100 TCD_{50} of Sindbis virus. Highest dilution of extract completely inhibiting CPE when 50% of cells in virus controls (VC) were affected (usually on 2nd or 3rd day) was taken to contain one unit of cyclopin. Cultures with 1 unit occasionally exhibited CPE a few days after virus controls which progressed to completion at a much slower rate than in the controls. Cultures containing 2 or more units exhibited no CPE during the entire period of observation (10-20 days).

Results. Concentration of cyclopin in different preparations. To date 18 different lots of cyclopin have been prepared from fungus grown in a variety of media at room temperature or 37°C. Concentration of cyclopin in different preparations ranged from 10 to 2560 units per ml. Incubation at room temperature in Sabouraud's broth containing 4% brown sugar gave titers as high or higher than other combinations tested. Assays of two lots prepared from fungus grown under these conditions were 1280 and 2560 units per ml.

On a few occasions after removal of the fungus, the growth media were assayed for cyclopin. Only low titers were found (3-5 units per ml).

Cell-toxicity. Cultures exposed to high concentrations of cyclopin (80-160 units) exhibited evidence of toxicity that consisted of rounding, slight swelling and increased granulation of cells. These effects were noted most

‡ Worthington Biochemical Co., Freehold, N. J.

§ Nutritional Biochemicals Corp., Cleveland, O.

TABLE I. Effect of Cyclopin on Cytopathogenicity and Replication of Representative Group A Arbor Viruses in Human Amnion Cell Cultures.

Virus*	Units cyclopin required to inhibit CPE	Units cyclopin added to cultures assayed for infectivity	Cultures with cyclopin		Virus controls	
			CPE	Inf†	CPE	Inf
Sindbis	1	64	0‡	NI	++++	6.6
"	1	16	0	NI	++++	6.6
"	1	2	0	NI	+++	6.2
WEE	.5	32	0	NI	++	3.2
Chickungunya	2	64	0	NI	++++	6.0

* Viral dose: 100 TCD_{50} inoculated immediately after addition of cyclopin.

† Infectivity assay of fluid from cultures removed on 4th day after inoculation in HA, expressed as log TCD_{50}/0.1 ml.

‡ 0 = Absence CPE.

NI = No infective virus demonstrated in undiluted fluid.

All cultures observed for a period of 7-10 days for CPE; infectivity assayed on 3rd or 4th day.

TABLE II. Effect of Cyclopin on Replication and Cytopathogenicity of Sindbis Virus in Human Amnion Cell Cultures.

	Test	Days after inoculation .5	1	3	5	7
Control	CPE	0	±	+++	++++	++++
	ICV	2.2	4.7	5.2	3.4	2.0
	ECV	3.2	5.2	7.2	7.2	5.8
Cyclopin*	CPE	0	0	0	0	0
	ICV	NT	.6	.5	0	0
	ECV	NT	.7	.4	0	0

Viral dose: 100 TCD_{50}. Viral assay in CE cells of pooled materials from 3 cultures; expressed as log TCD_{50}/0.1 ml.

NT: Not tested. CPE: Cytopathic effect; avg of readings in all cultures available on day indicated. ECV: Extra-cellular virus. ICV: Intra-cellular virus.

0 = No CPE or infectivity detected.

* 16 units of cyclopin added at time virus inoculated.

prominently at the margins of the monolayer. Chick embryo cells appeared to be more affected than HA cells. Cultures exhibiting such changes, however, metabolized normally as indicated by undiminished acid production and capacity to support replication of viruses not inhibited by cyclopin. Inactivation of cyclopin by trypsin (Table VIII) did not significantly reduce toxicity. This finding suggests that accompanying contaminating material rather than cyclopin itself was responsible for the toxic effects.

Inhibition of representative Group A arboviruses by cyclopin in HA cell cultures. Effect of cyclopin was tested on 3 Group A arboviruses: Sindbis, Western Equine Encephalitis, and Chickungunya virus, in HA tissue cultures. Results, summarized in Table I, show that CPE and replication of all these agents were completely suppressed when the inhibitor was added in amounts ranging from 2 to 64 units along with 100 TCD_{50} of virus. Cultures were observed over a period of 7-10 days for CPE and fluids removed on the 3rd or 4th day were assayed for infectivity.

To analyze further effect of cyclopin on growth of Sindbis virus, assays of infectivity of fluids and cellular components were carried out separately at varying intervals after inoculation when extent of CPE was also recorded. In this experiment a series of HA cultures inoculated with 100 TCD_{50} of Sindbis virus were kept as virus controls. In another series 16 units of cyclopin were added to each culture which was immediately inoculated with 100 TCD_{50} of virus. On days 1, 3, 5 and 7 intra-cellular and extra-cellular virus present in 3 cultures from each series was assayed in CE cultures. Results are presented in Table II. Traces of virus found in

TABLE III. Effect of Cyclopin on Replication of Representative Group A and B Arboviruses in Chick Embryo Cell Cultures.

Virus	Viral dose TCD_{50}	Units cyclopin required to inhibit CPE	Units cyclopin added to cultures assayed for infectivity	Cultures with cyclopin		Virus control	
				CPE*	Inf	CPE	Inf
Sindbis	100,000	8	—	0	NT	++++	NT
"	1,000	4	16	0	.8	++++	7.2
"	100	4	32	0	NI	++++	7.8
"	100	4	4	0	3.8	++++	7.8
WEE	100,000	4	—	0	NT	++++	NT
West Nile virus	10,000	4	32	0	1.2	++++	7.2
" " "	10,000	4	8	0	4.2	++++	7.2

* CPE in cultures assayed for viral infectivity.

Viral assay in CE cells; expressed as log TCD_{50}/0.1 ml.

0 = Absence CPE. NT = Not tested. NI = No infective virus demonstrated in undiluted fluid.

All cultures observed over a period of 7 days for CPE. Infectivity assayed on the 3rd day for Group A and on the 5th day for Group B arboviruses.

cultures with cyclopin may possibly represent that portion of the original viral inoculation which remained viable without replicating since assays were done in chick cell cultures and 100 TCD_{50} of Sindbis virus for amnion cultures is approximately equivalent to 10,000 TCD_{50} for chick cell cultures(7).

Inhibition of representative arboviruses Groups A and B in CE cell cultures. Effect of cyclopin on 2 arboviruses of Group A (Sindbis, WEE) and on one of Group B (West Nile) was tested in this system. Table III shows that when large doses of virus were used CPE was suppressed and viral replication markedly inhibited. With smaller doses of virus and higher concentrations of cyclopin both phenomena were completely inhibited. In another experiment inhibition of multiplication and hemagglutin production by arboviruses Groups A and B (Sindbis and West Nile) was demonstrated. Results are presented in Table IV.

Effect on other viruses. No significant inhibition of CPE or viral replication was noted in cultures infected with poliovirus type 1, ECHO 11, adenovirus type II, herpes simplex virus, Coxsackie virus A9, B2 and B5 when virus and cyclopin were added simultaneously. However, when cells were treated with cyclopin 16-24 hours before viral inoculation, a varying degree of temporary inhibition of CPE and viral replication was observed with all the agents listed in Table V except herpes simplex virus.

"Therapeutic" effect on established infection. A series of HA cell cultures were inoculated with 100 TCD_{50} of Sindbis virus. One series was kept as virus controls and to the others 16 units of cyclopin were added at 2, 4, 8 and 16 hours after infection. CPE appeared at about 48 hours in the controls and reached 4+ by the 4th day. At this time none of the cultures with cyclopin showed CPE.

To determine extent of replication when cyclopin was added after the virus the following experiments were performed: A series of HA tubes were inoculated with 100 TCD_{50} of Sindbis virus. Twelve hours later all tubes were washed 5 times with 5 ml medium 199. The fifth washing was assayed for infectious virus; none was detected. To each tube of one series, 1 ml maintenance medium was added and these cultures kept as virus controls; to each of the other series, 1 ml medium containing 16 units of cyclopin was added. Every day thereafter fluids and cellular extracts prepared by pooling materials from 3 tubes of each series were assayed for infectivity. The experiment was repeated adding cyclopin 24 hours after viral inoculation. Tables VI and VII summarize the results which indicate that the amount of extracellular virus produced by cultures receiving cyclopin 12 and 24 hours after infection represented only a small fraction (0.0001-0.001%) of that replicated in control cultures without inhibitor.

Pre-treatment of tissue cultures. When HA cultures were pretreated with cyclopin 16 to

TABLE IV. Effect of Cyclopin on Replication and Hemagglutinin Production of Sindbis and West Nile Virus.

Virus	Viral dose TCD_{50}	Tissue culture	Units cyclopin required to inhibit CPE	Units cyclopin added to cultures assayed for infectivity	Cultures with cyclopin			Virus control		
					CPE	Inf	HA†	CPE	Inf*	HA†
Sindbis	100	H. amnion	1	16	0	NI	0	++++	6.2	1:640
"	100	" "	1	4	0	NI	0	++++	6.2	1:640
West Nile	1000	CE cells	8	32	0	NI	0	++++	7.2	1:320
" "	1000	" "	8	8	0	NI	0	++++	7.2	1:320
" "	100	" "	4	16	0	NI	0	++++	7.2	1:320
" "	100	" "	4	8	0	NI	0	++++	7.2	1:320

0 = Absence CPE or hemagglutinin titer. NI = No infective virus demonstrated in undiluted fluid.
All cultures observed over a period of 10 days. Infectivity and hemagglutination titer assayed on 4th day in Sindbis and on 6th day in West Nile virus inoculated cultures.

* Viral infectivity, expressed as log TCD_{50}/0.1 ml.

† HA = Hemagglutinin titer.

24 hours before inoculation of 100 TCD_{50} of Sindbis virus it was found that 0.25 to 0.5 unit completely suppressed CPE. When cyclopin was removed before viral inoculation from cells thus treated, no inhibition was observed unless high concentrations of the inhibitor had been applied (32 units or more). When cultures had been in contact with such high concentrations for 16-24 hours before inhibitor was removed no CPE was noted. In these experiments cyclopin was removed by washing 5 times with 5 ml aliquots of medium 199.

Mode of action. A priori it is possible that the inhibitor may prevent viral multiplication by directly inactivating the free virus particles or by blocking one of the following steps essential in synthesis of new virus: a) adsorption; b) penetration; c) replication; d) release. Experiments were performed to determine at which of these possible points inhibition was affected. Lack of a direct inactivating effect of cyclopin was shown in the following manner. A mixture of equal volumes of Sindbis virus (100 TCD_{50}/0.1 ml) and maintenance medium containing cyclopin (32 units/ml) was prepared and incubated at 37°C for 2 hours. Aliquots of 0.2 ml of the mixture were added to each of 3 HA cell cultures. After 2 hours at 37°C cultures were washed 5 times with 5 ml medium 199 and 1 ml of maintenance medium was then added to each tube. Control cultures without cyclopin were similarly treated. After incubation at 37°C CPE appeared simultaneously in all cultures.

That adsorption and penetration of Sindbis virus is not affected by the inhibitor is indicated by results of the following experiment: A batch of HA cell cultures was divided into 4 groups of 3 each which were treated as follows:

Group 1: Inoculated with 1000 TCD_{50} of Sindbis virus.

Group 2: To each culture 16 units of cyclopin were added and then 1000 TCD_{50} of Sindbis virus.

Group 3: Cultures were treated like those of Group 2. After incubation at 37°C for 2 hours they were washed 5 times with 5 ml of medium 199. One ml of maintenance media

TABLE V. Effect of Cyclopin on Replication of Enteroviruses, Herpes Simplex, and Adenovirus.

Virus	Culture for test	Units cyclopin added*	Culture for viral assay	Cultures with cyclopin		Controls	
				CPE	Inf†	CPE	Inf†
Polio virus I	HA	32	MK	±	4.2	++++	6.2
Idem	HA	8	MK	++	5.6	++++	6.2
ECHO 11	HA	32	MK	++	5.8	++++	6.8
Coxsackie A9	MK	32	MK	+++	5.8	++++	6.2
" B2	MK	32	MK	++	5.2	+++	5.8
" B5	MK	32	MK	+++	5.6	++++	6.2
Adenovirus Type II	HA	32	HA	±	1.2	+++	2.2
Herpes simplex	HA	32	—	++++	NT	++++	NT

* Cultures were treated with cyclopin 16-24 hr before viral inoculation.
† Infectivity titer is expressed as log TCD_{50}/0.1 ml.
NT = Not tested.
Cultures inoculated with enteroviruses and herpes simplex observed over a period of 6 days for CPE and viral infectivity assayed on 3rd day. In cultures inoculated with adenovirus type II observed over a period of 2 wk, viral infectivity assayed on 7th day.

TABLE VI. Effect of Cyclopin on Replication of Sindbis Virus when Added 12 Hours After Viral Inoculation.

Test	Days after inoculation of virus							
	0.5	1	2	3	4	5	6	7
				Control				
CPE	0	±	+	+++	++++	++++	++++	++++
ICV		4.3	5.2	5.4	5.2	3.2	NT	2.4
ECV	*	5.6	7.2	7.2	7.6	7.2	NT	5.6
				Cyclopin				
CPE	0	0?	0?	0	0	0	0	0
ICV		.7	.8	.2	.6	.2	NT	.2
ECV	†	2.4	3.2	2.2	1.3	1.6	NT	.6

* All cultures washed 5× with 5 ml of Medium 199, and 1 ml of maintenance medium added.
† *Idem*, added maintenance medium containing 16 units of cyclopin.
Test cultures: HA cells. Viral assay in CE cells: Titer expressed as log TCD_{50}/0.1 ml. Viral inoculum: 100 TCD_{50}.
0 = Absence CPE.
NT = Not tested.

TABLE VII. Effect of Cyclopin on Replication of Sindbis Virus when Added 24 Hours After Viral Inoculation.

Test	Days after inoculation of virus							
	0.5	1	2	3	4	5	6	7
				Control				
CPE	0	±	+	+++	++++	++++	++++	++++
ICV			4.4	4.2	4.2	3.4	NT	2.2
ECV		*	6.2	7.2	7.8	7.2	NT	4.8
				Cyclopin				
CPE	0	±	+	±	0?	0	0	0
ICV			4.2	2.4	2.6	2.8	NT	1.8
ECV		†	4.2	4.6	4.6	2.4	NT	2.6

* All cultures washed 5× with 5 ml of Medium 199, and 1 ml of maintenance medium added.
† *Idem*, added maintenance medium containing 16 units of cyclopin.
Test cultures: HA cells. Viral assay in CE cells: Titer expressed as log TCD_{50}/0.1 ml.
0 = Absence CPE.
NT = Not tested.

TABLE VIII. Some Physico-Chemical Properties of Cyclopin.

	Exp I	Exp II	Exp III
Cyclopin alone	80*	160	320
" + trypsin	10	20	40
" + RNA-ase	80(10γ)†	80(100γ)†	NT
" + DNA-ase	NT	160(100γ)†	NT
" + protamin	80	160	NT
" , 37°C × 3 hr	80	160	320
" , 56°C × 3 "	<10	<10	NT
" , 100°C × 5 min	<10	NT	NT
" pH 2.4	<10	NT	NT

* Units/ml at end of test period.
† Concentration of RNA-ase or DNA-ase used.
NT = Not tested.

was then added to each. The last washing was tested for viral infectivity. None was detected.

Group 4: Cultures were treated like those of Group 3. After washing, 1 ml of 1:20 dilution of Sindbis antiserum in maintenance medium was added to each. Cultures were kept at room temperature for 2 hours, washed 5 times with 5 ml medium 199, and 1 ml of maintenance medium was added to each tube.

CPE appeared in all cultures of Groups 1, 3 and 4 but not in those of Group 2. Viral multiplication occurring in Group 3 indicated that adsorption of virus was not prevented by cyclopin. Since multiplication also occurred in Group 4, where cell-adsorbed virus that had not entered the cells would presumably have been neutralized by the antiserum present, it may be inferred that penetration of the agent was not prevented by the presence of cyclopin. Results of the experiments shown in Table II demonstrate that release of the Sindbis virus from HA cells is also not altered by cyclopin, since in its presence titer of intra-cellular virus at no time exceeds a barely detectable level.

Physico-chemical properties of cyclopin. Cyclopin was treated with trypsin, RNA-ase, DNA-ase, and protamin, respectively, and also subjected to different temperatures according to procedures previously employed in the study of a viral inhibitor derived from a bacterial source(8). Results of 3 experiments summarized in Table VIII show that most or all of the activity is destroyed by treatment with trypsin and moderate heating and exposure to pH of 2.4. Nucleases and protamin were without effect. These findings suggest that the active factor may be a protein or protein derivative. In 3 different experiments cyclopin of known unitage was placed in a cellophane bag and dialyzed against bovine amniotic fluid medium at 4°C. Activities of the fluid inside and outside the bag were then assayed. Results showed that 50-70% of the active factor passed through the dialyzing membrane. This finding along with results of trypsinization favor the view that cyclopin, in part at least, is a protein derivative.

Discussion. Although many antibiotics derived from fungi are effective against bacteria, we are aware of only 3 antiviral substances of mycologic origin that have been reported in the literature. Schabel and his coworkers(9) described Netropsin, an antibiotic from culture filtrates of *Streptomyces netropsis* that exhibited a therapeutic effect on experimental vaccinial infection of mice. Helenin, a substance derived from *Penicillium funiculosum* was found by Shope(10) to have antiviral properties in mice infected with Columbia SK encephalitis or Semliki Forest virus. Cochran and his associates(11) also demonstrated that Helenin had a prophylactic effect in monkeys against a paralytigenic strain of poliovirus type I, but no therapeutic action when administered after viral inoculation. Shope(10) postulated that Helenin acted either by blocking viral invasion of the central nervous system or by inhibiting viral replication in host cells. Powell and his coworkers(12) described an antiviral substance (M5-8450) derived from *Penicillium stoloniferum* that protected mice against MM and Semliki Forest viruses. In these animals a therapeutic effect was detectable only if given within a few hours after viral inoculation. Hull and Lavelle(13) showed that M5-5450 inhibited cytopathic effect of types I, II, and III polioviruses when tested in monkey testicular cell cultures.

Perhaps the most striking feature of cyclopin as compared with these other fungal derivatives is the limitation of activity to a single group of related viruses. Thus our experiments show that it effectively prevents multiplication and cytopathic effects of arbo-

viruses Groups A and B, but not those of several other unrelated agents. Certain of these unrelated viruses are slightly and temporarily inhibited when the cells are pretreated with cyclopin but, in sharp contrast to the arbo group, are not affected when cyclopin is added at the time of viral inoculation. Whether this transitory effect is specific or attributable to temporary impairment of cellular function remains to be determined.

Worthy of comment also is the demonstration that cyclopin, in an unexplained manner, interferes with replication of these agents within the cells rather than by inactivating them on contact or by preventing their adsorption or release from the cells. One cannot compare the mode of action of cyclopin with the other fungal derivatives mentioned above, since no reports of studies on the mechanism of action of the latter have appeared.

The capacity of cyclopin to arrest viral synthesis in cell cultures, even when added 12 to 24 hours after the virus, is of interest since very few substances with this activity are known. However, it should be emphasized that the original inoculum was only 100 TCD_{50}. Notable among other antiviral substances with such properties are 2-(α-hydroxybenzyl)-benzimidazole(14) and IDUR (15), the former inhibiting the CPE of polio virus and the latter inhibiting the CPE of herpes simplex and vaccinia under these conditions.

Cyclopin in its mode of action and in certain of its physical and chemical activities resembles interferon(16). It differs, however, in limitation of activity to the arbovirus groups, as so far determined, in its instability at pH 2.4, and in its dialyzability. It is also noteworthy that the viral inhibitory effect of cyclopin is not tissue specific, since our experiments show that cyclopin effectively inhibits replication of arboviruses in both human and chick cell cultures. The results, however, suggest that human cells in culture are more sensitive to the activity of cyclopin than those of the chick embryo as demonstrated by the difference in minimal dosage required to suppress cytopathic effect in these two systems.

Experiments with mice of different ages are in progress to determine whether cyclopin may prevent or modify infection with selected members of the arbovirus group.

Summary. A constituent of *penicillium cyclopium* has been found to have antiviral properties. Preliminary analyses suggest that it is a protein derivative. This factor, provisionally designated "Cyclopin," inhibits *in vitro* both cytopathic effect and replication of representative species of Groups A and B arboviruses even when added after infection is established. Under comparable conditions it does not significantly inhibit cytopathic changes induced by certain enteroviruses, adenovirus type II, and herpes simplex virus.

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1. Milovanovic, M. V., Enders, J. F., Mitus, A., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 120.

2. Katz, S. L., Milovanovic, M. V., Enders, J. F., *ibid.*, 1958, v97, 23.

3. Frothingham, T. E., *ibid.*, 1959, v100, 505.

4. Enders, J. F., *ibid.*, 1953, v82, 100.

5. Reed, L. J., Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

6. Clarke, D. H., Casals, J., *Am. J. Trop. Med. and Hyg.*, 1958, v7, 561.

7. Gresser, I., personal communication.

8. Carver, D. H., Naficy, K., PROC. SOC. EXP. BIOL. AND MED., 1962, v111, 356.

9. Schabel, F. M., Laster, W. R., Brockman, R. W., Skipper, H. E., *ibid.*, 1953, v83, 1.

10. Shope, R. E., *J. Exp. Med.*, 1953, v97, 601.

11. Cochran, K. W., Francis, T., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 230.

12. Powell, H. M., Culbertson, C. G., McGuire, J. M., Hoehm, M. H., Baker, L. A., *Antibiotic and Chemotherapy*, 1952, v2, 432.

13. Hull, R. N., Lavelle, J. M., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 787.

14. Tamm, I., Bablanian, R., Nemes, M. M., Shunk, C. H., Robinson, F. M., Folkers, K., *J. Exp. Med.*, 1961, v113, 625.

15. Kaufman, H. E., Maloney, E. D., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 4.

16. Isaacs, A., Lindenman, J., *Proc. Roy. Soc. London*, 1957, sB 147, 258.

Received June 20, 1963. P.S.E.B.M., 1963, v114.