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Observations on Antiviral Activity of Netropsin.* (20246)

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Many antibiotics and other substances which have been found to be active against viruses of the psittacosis-lymphogranuloma group also have antibacterial and/or antirickettsial activity. On the basis of such chemotherapeutic experiments Horsfall has pointed out(1) that perhaps the psittacosis-lymphogranuloma group should not be classified among the "true" viruses. The classification of the psittacosis-lymphogranuloma group as the family Chlamydozoaceae in the order Rickettsiales(2) supports this view. It is for treatment of infections with microorganisms of the order of Virales that active therapeutic substances are most conspicuously lacking(1,3-5). Some of the structural analogs of purine and pyrimidine constituents of nucleic acid have been reported to inhibit the multiplication of vaccinia virus in chick-embryo tissue culture(6), and some 5-phenoxypyrimidines, only moderately active inhibitors of vaccinia virus in chick embryonic tissue culture, have been shown to have a significant protective effect against intranasally or intracerebrally inoculated vaccinia virus in mice(7). One of the purine analogs, 2,6-diaminopurine, has been shown to increase the survival rate in mice infected with Russian Spring-Summer Encephalitis (RSSE) virus via the intraperitoneal route(8). This compound also inhibits RSSE virus in tissue culture(9). Two groups of investigators (10,11) have reported that several variously substituted thiosemicarbazones have *in vivo* antivaccinial activity. We have essentially confirmed these observations in the treatment of vaccinial infections in mice with substituted thiosemicarbazones(12). With the exception of the examples listed, chemotherapy of members of the order Virales has not been markedly successful(4).

In a search for effective antiviral agents being carried out in this laboratory we have tested numerous antibiotics and known tumor-inhibiting compounds for antiviral activity. Among the antibiotics tested was "Netropsin,"† isolated by Finlay *et al.*, from culture filtrates of *Streptomyces netropsis*(13). The

* This work was carried out under a contract from the Chemical Corps Biological Laboratories, Camp Detrick, Frederick, Md.

TABLE I. Effect of Netropsin on Vaccinial Infection in Mice. Duration of treatment 5 days.

Treatment	Dosage (mg/kg)	Days of death 5	6	7	8	9	10*	14	Survivor, total	% survivors	Challenge survivors, total
N†	35				1	2		1	16/20	80	16/16
C			6	4	4	1			4/19	22	3/4
N	35				1				39/40	98	35/35
C			5	3		1	1		10/20	50	10/10
N	35				2		1		17/20	85	12/12
C			9	6	6	1			3/25	12	2/2
N	30						1		15/16	94	15/15
C		2	3	5	2	1			6/19	32	6/6
Totals N					4	2	1	1	88/96	92	78/78
C		2	23	18	12	4	1		23/83	28	21/22

* No deaths 11, 12 and 13th day.
† N = Netropsin; C = Controls.

present report deals with the effect of netropsin on vaccinial infections in mice.

Experimental. The virus used was the WR strain of mouse neurotropic vaccinia virus obtained from the American Type Culture Collection. The LD_{50} of this virus for intracerebrally inoculated mice is approximately 0.03 ml of a $10^{-4.5}$ dilution of virus infected mouse brain. Mice used were white Swiss, obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Mixed sexes weighing approximately 15 g apiece were employed in all experiments. Aqueous solutions of netropsin undergo change on storage, evidenced by changes in the chromatogram, ultraviolet absorption, antibiotic activity and toxicity(14). Mice were inoculated intracerebrally with one LD_{50} to one LD_{90} of virus and treatment was begun 30 to 60 minutes later. One-half a daily maximum tolerated dose (M.T.D.) of netropsin in aqueous solution was given intraperitoneally to each mouse morning and late afternoon for 5 days. Control mice were injected with distilled water at the same time the drug was given to the treated mice. Fourteen days after the first virus inoculation, all survivors were challenged intracerebrally with about 1000 LD_{50} of homologous virus, the challenge inoculation being given in the opposite cerebral hemisphere from the primary inoculation. The M.T.D. of netropsin for mice was taken as ⅓ the intraperitoneal LD_{50} dose. This was found to be 30-35 mg/kg/day for 5 days.

† Netropsin was supplied by Chas. Pfizer and Co., Brooklyn, N. Y.

Table I summarizes a series of experiments in which mice infected with vaccinia virus via the intracerebral route were treated with netropsin.

It will be noted that netropsin markedly increased the survival rate of mice inoculated with vaccinia virus. It also prolonged the life span of those treated mice which died.

Mice which survived the initial inoculation of vaccinia virus almost invariably resisted challenge with homologous virus. The following experiment indicated that this immunity resulted from virus infection and not from the antigen present in the original inoculum. Mice were inoculated as in the experiments of Table I, but using a mouse brain suspension of virus which had been heated at 55°C for one hour on 2 occasions, 17 days apart. When challenged 14 days after the original inoculation with about 1000 LD_{50}, given intracerebrally, nearly all the mice died with typical signs of vaccinial infection. These observations essentially confirm previous reports that vaccinial immunity is not readily induced by inoculation with other than large amounts of inactive virus(15) and survivors of vaccinial infections resist challenge with multiple lethal doses of virus(16). These observations indicate that the increase in survival rates of netropsin-treated over control mice (Table I) is due to the activity of the drug in animals with active virus infections. Netropsin, administered at the M.T.D. for a period of days, results in marked weight loss in treated mice.

To determine whether the netropsin was protecting infected mice by some mechanism

TABLE II. Comparison of Starvation and Netropsin Treatment in Mice Infected with Vaccinia Virus.

Treatment	Days after inoculation with virus				Days of death						Survivor, total	% survivors	Resisted challenge, total
	0	2	6	8	5	6	7	8	9	10			
	Avg wt (g)												
Netropsin, 30 mg/kg/day, 5 days	14.9	13.7	11.8	11.9						1	18/19	95	15/15
Starvation	15.1	13.4	12.2	10.7	2	5	7	3	1		2/20	10	1/1
Controls	15.1	13.6	12.6	13.0	2	3	5	2	1		7/20	35	6/6

associated with weight loss the following experiment was carried out. Sixty mice were inoculated with vaccinia virus via the intracerebral route. They were then separated into 3 groups of 20 mice each. One group was treated with netropsin; a second group was left untreated and food was restricted but water was given *ad libitum;* and a third group was given control injections of water. All three groups were weighed at intervals and average weights were recorded. Results of this experiment, listed in Table II, indicate that weight loss similar to that seen in netropsin-treated mice does not account for the protective action attributed to the drug.

The effect of netropsin on intradermal vaccinial infections in rabbits was tested. Serial 10-fold dilutions of virus were inoculated intradermally into rabbits and M.T.D. amounts of netropsin were injected intraperitoneally into treated animals on a schedule of one-half daily dose twice a day for 5 days. No difference was noted in the skin infections between the control and drug-treated rabbits. It should be pointed out, however, that the M.T.D. of netropsin for rabbits is only about 20% of the M.T.D. for mice on a body weight basis, and therefore the rabbits did not receive as intensive drug treatment as did the mice.

The effect of netropsin on feline pneumonitis virus, influenza virus (Type A), Western equine encephalomyelitis virus (California strain), and poliomyelitis virus (Lansing strain) infections in mice was also tested and the drug showed no effect on any of these virus infections in mice.

We are at present testing the effect of this drug on variola virus infections. The details of these and other tests for antiviral activity of a number of other antibiotics and other materials will be published elsewhere.

Summary. Netropsin, an antibiotic from culture filtrates of *Streptomyces netropsis,* has marked therapeutic effect on experimental vaccinial infections (WR strain) in intracerebrally inoculated mice. It is ineffective against infections in mice with the viruses of feline pneumonitis, influenza, Western equine encephalomyelitis, and poliomyelitis.

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