antigen(22). Using the known properties, such as host range, mode of transmission, systemic infection and predilection for nervous tissue of these 5 viruses as a possible pattern of characteristics, it would seem that the eastern and western equine encephalomyelitis viruses would answer the requirements but experiments showed that these agents had no oncolytic effect on RPL-12. Any hopes engendered by the data presented in this report must be tempered by the realization that those agents which were found to be effective are impractical for use in public health or veterinary medicine. In addition, it should be emphasized that it is probably true in chicks, as has been shown for mice(23), that any one viral agent is not effective against all types of neoplasia. It is apparent, therefore, that the search for agents which may be employed with safety must be continued along broad lines and it is possible that effective viral agents may be found which have neither the antigenic nor pathogenic characteristics in common with the neurotropic group reported above.

Conclusions. 1. RPL-12, a transplantable lymphoid tumor of chickens, can be caused to regress, without any apparent damage to the host, by superimposed inoculations of Russian Spring-Summer, West Nile, Japanese B encephalitis, St. Louis encephalitis or louping-ill viruses.

2. Chickens which have been inoculated with the RPL-12 tumor followed by a superimposed infection with any of the 5 viral agents are rendered immune, as a rule, to challenge by homologous tumor even though the original inoculation with tumor may not have produced a palpable tumor.

3. The infective titer of RSS virus in infected tumor tissue is equal to that of infected mouse brain.

4. RSS virus obtained from infected mouse brain has a high oncolytic action when introduced into the tumor area but is less effective when it is introduced at a distance from the tumor.

5. RSS virus obtained from infected RPL-12 tumor has a high oncolytic action when introduced either in the tumor area or at a distance from the tumor.

The authors wish to express their appreciation to Miss Margaret Batstone for her able technical assistance during the course of these studies.

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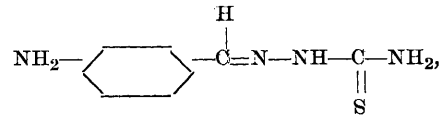
Activity of *p*-Aminobenzaldehyde, 3-Thiosemicarbazone on Vaccinia Virus in the Chick Embryo and in the Mouse. (17652)

DOROTHY HAMRE, JACK BERNSTEIN, AND RICHARD DONOVICK.
From the Squibb Institute for Medical Research, New Brunswick, N. J.

The recent addition of the thiosemicarbazones to the list of therapeutic agents for the treatment of tuberculosis(1-3) led us to investigate some of these compounds for possible activity against other pathogenic microorganisms including some of the viruses. Because it was the most soluble compound in our collection of thiosemicarbazones at the time this work was undertaken, the *p*-aminobenzaldehyde 3-thiosemicarbazone (MC2343),



was selected for first tests against vaccinia virus.

Vaccinia tests in chick embryos. In this test,

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TABLE I.
Treatment of Embryonated Eggs Infected Via the Yolk Sac with Vaccinia Virus.

Exp. No.	Treatment	Dose per egg, mg	LD_{50} units of vaccinia in inoculum: 340 AST*	340 Dead Total	170 AST	170 Dead Total	85 AST	85 Dead Total
1	Saline (control)	—	96	6/6	119	5/5	109	6/6
	MC2343	0.06	128	6/6	151	6/6	191	*3*/6
	Neomycin	10	112	6/6	122	6/6	138	6/6
	γ-Globulin	18.5	121	6/6	126	6/6	139	6/6
2	Saline	—	103	12/12	104	12/12	130	12/12
	MC2343	.06	170	*10*/11	203	*8*/11	174	*5*/11
	"	.03	151	12/12	167	*11*/12	184	*6*/12
3	Saline	—	94	6/6	98	6/6	98	6/6
	10% triethylene glycol	—	90	6/6	113	6/6	113	6/6
	MC2343	.06	123	6/6	151	6/6	181	6/6
	"	.03	158	6/6	171	6/6	175	6/6
	"	.015	133	6/6	135	6/6	167	6/6
	" air sac	.05	127	6/6	160	*4*/6	147	6/6
	Myvizone (in TEG)	.01	135	6/6	114	6/6	158	6/6

* Average survival time in hours of eggs that died. Eggs surviving beyond the 19th day of incubation were not included in these averages.

6-day-old chick embryos were infected via the yolk sac with approximately 85, 170, and 340 LD_{50} units of a frozen vaccinia pool (New York Board of Health strain of vaccinia). Usually 6 eggs were infected with each dose for each compound to be tested and for untreated controls. Thirty minutes after infection the eggs were treated via the yolk sac; control eggs being given saline. The eggs were incubated at 36.5°C, candled twice daily (except on Saturday and Sunday when they were candled once daily) and survival time of the eggs recorded. Chorioallantoic membranes of dead eggs were examined for lesions to establish the cause of death as due to vaccinia. Deaths of eggs prior to 70 hours after injection were usually non-specific.*

The results of 3 experiments with MC2343 are given in Table I. In the first experiment, a partially purified preparation of neomycin(4) and a γ-globulin fraction from vaccinia-immune calf serum are also included. These latter two substances both caused a significant delay in death of the eggs ($P = 0.01$), but no eggs survived beyond the 14th day of incubation; while 3 out of 6 eggs infected with 85 LD_{50} units of vaccinia and treated with 0.06 mg of MC2343 survived through the 19th day of incubation. In Experiment 2, the results with MC2343 at 0.06 and 0.03 mg per egg were both promising, producing a significant delay in death of the eggs that died, and protecting 33% of those treated with 0.06 mg and 19% of those treated with 0.03 mg. In the third experiment, 0.06 mg, 0.03 mg and 0.015 mg all caused significant delays in death ($P = <0.01$) but none of the eggs survived. This variation in response to MC2343 from experiment to experiment may be due either to variation in the vaccinia content among the ampules in the frozen pool, or to variations in the response of the eggs to infection with the virus.

Since the eggs are treated by the same route as that used for infection, the possibility of a virucidal rather than a chemotherapeutic action existed. However, in another experi-

* The tests described here are modifications of tests in eggs and in mice with vaccinia which will be reported in a publication in the near future by F. P. O. Nagler and Geoffrey Rake. The details of the present egg test, including reasons for using this experimental design and its statistical analysis (for which we are indebted to K. A. Brownlee), are also being prepared for publication.

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TABLE II.
Treatment of Mice Infected Intranasally with Vaccinia Virus.

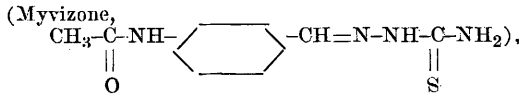
Treatment	Route	Dose, mg/kg daily	No. of mice dying day after infection							AST,* days	Dead	% surviving
			4	5	6	7	8	9	10		Total	
Saline	SC	—		2	4	4				6.2	10/10	0
γ-Globulin	''	100			3	3	1			6.7	7/10	30
MC2343	''	40				6		2	2	8.0	10/10	0
Saline	''	—			5	4	1			6.6	10/10	0
MC2343	''	40				3	6			7.6	9/10	10
''	per os	40				1	5	1	1	8.2	8/10	20
Myvizone	''	48			1	2	7			7.6	10/10	0

* Surviving mice were omitted from calculation of average survival time.

ment, treatment with 0.06 mg delayed for 4 hours after infection gave results similar to those reported above in which treatment followed 30 minutes after infection. In addition, treatment with 0.05 mg of MC2343 via the air sac 30 minutes after infection produced a significant delay in death ($P = <0.01$) and 2 out of the 18 eggs survived (experiment 3, Table I).

Other thiosemicarbazones are being tested against vaccinia in eggs; however, the insolubility of most of these compounds in water necessitates the use of 10-25% aqueous triethylene glycol as solvent. This solvent was chosen on the basis of its low toxicity for embryonated eggs when given via the yolk sac and its lack of significant activity against vaccinia virus in this test.

Results of one experiment with *p*-acetamido-benzaldehyde thiosemicarbazone (Myvizone,



), given in Table I, experiment 3, indicate that this compound also has activity against vaccinia virus. Larger doses of Myvizone could not be tested because of its high toxicity for embryonated eggs. The approximate LD_{50} of Myvizone was 0.02 mg per egg, compared with MC2343 which was 0.125 mg per egg.

Vaccinia tests in the mouse. MC2343 was then tested in mice (12-14 g CFl strain) infected intranasally with 0.05 ml of a 10% mouse brain suspension of vaccinia. This suspension contained about 10^7 virus units per ml according to the pock count on the chorioallantoic membrane. The mice were treated with 0.5 mg (40 mg/kg) of MC2343 subcutaneously 30 minutes after infection and daily thereafter for 4 days. As a control active substance, 13 mg (100 mg/kg) of γ-globulin was given subcutaneously for the same number of doses. Control mice received saline as treatment. Results are given in Table II.

Although none of the mice treated with MC2343 survived, the delay in death of these mice was considered as confirmation of the activity of this compound against vaccinia virus in another host.

In the second experiment in Table II MC2343 was given subcutaneously to one group of mice and by mouth by catheter to another group in order to compare it with Myvizone which was given by mouth. Both drugs, when administered orally, were suspended in 5% acacia. The schedule of treatment was the same as for the first experiment.

Myvizone produced an average delay in death of the mice comparable to that produced by MC2343 subcutaneously, but none of the mice survived whereas 10% of those treated subcutaneously and 20% of those treated by mouth with MC2343 survived beyond the 25th day. These results indicate a higher activity against vaccinia virus for MC2343 than with Myvizone; however, further experiments are now in progress and a final conclusion on rank of activity must be deferred. Other thiosemicarbazones are also being tested against vaccinia virus in the mouse.

MC2343 was tested and found to be inactive, at a dose of 0.06 mg per egg via the yolk sac against about 100 LD_{50} doses of the agent of meningopneumonitis and against about 100 LD_{50} doses of the vole rickettsia (Baker). At 40 mg/kg, using the same treatment schedules as for the vaccinia experiments in mice, MC2343 was inactive against influenza virus (Shope swine influenza strain V15) in mice infected intranasally.

Summary. *p*-Aminobenzaldehyde, 3-thiosemicarbazone (MC2343) caused a significant delay in death and survival of a small percentage of chick embryos and mice infected with vaccinia virus.

At the concentrations tested this compound was inactive against the agent of meningopneumonitis, the vole rickettsia (Baker) and swine influenza virus.

Preliminary results indicated that the *p*-acetamido analogue (Myvizone) was also active against vaccinia virus.

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Excretion of Poliomyelitis Virus by Monkeys Used for Testing Material of Human Origin.* (17653)

ALEX J. STEIGMAN† AND ALBERT B. SABIN.

From The Children's Hospital Research Foundation, Department of Pediatrics, University of Cincinnati, College of Medicine.

The variables which determine excretion of poliomyelitis virus in the stools of infected animals cannot as yet be clearly defined.(1) It was deemed desirable, therefore, to collect additional data on the excretion of virus by monkeys which develop paralysis following inoculation with material of human origin.

Methods. As part of another investigation,(2) there were available rhesus and cynomolgus monkeys which had developed paralysis following inoculation with material from a considerable number of individual patients with poliomyelitis. Each monkey was inoculated intracerebrally with the extract of throat swabs, intraabdominally with ether-treated stool extract, and intranasally with untreated stool suspension of the same patient. During the course of the nasal instillations, it was obvious that most of the 1 ml of stool suspension, which was put into each nostril each day, was being swallowed. The presence of typical poliomyelitis lesions in the olfactory bulbs of the monkeys which became paralyzed indicated that the stool suspensions which were being instilled probably contained active virus. Such animals could, therefore, be regarded as having received active virus by the oral route, the nasal route, the intraabdominal route and possibly, also, the intracerebral route. The monkeys which received the human material were killed by chloroform anesthesia on the first day of paralysis. The colon was excised aseptically and its entire contents were removed without disturbing the mucosa and stored in the frozen state in a dry ice box. In only one instance were the contents from 2 monkeys, which received material from the same patient, pooled for testing. Otherwise, the colon contents from each monkey were tested individually. After thawing the frozen material at 37°C, it was ground in a mortar with sufficient distilled water to make approximately a 10% suspension. This suspension was allowed to sediment in long tubes for 1 hour in the refrigerator; the sediment was retained for subsequent nasal instillation in the recipient monkeys, while the supernatant liquid was treated with anesthetic ether, which was added in sufficient quantity to yield a final concentration of 20% by volume. After

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

† Senior Fellow in Pediatrics, National Research Council.

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