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Effect of Aliphatic Oxime and Isatin Thiosemicarbazones on Vaccinia Infection in the Mouse and in the Rabbit.* (20690)

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Mice inoculated intracerebrally with vaccinia virus and treated with certain thiosemicarbazones containing either benzene or heterocyclic groups are protected against the virus (1-3). The concentrations of virus in the brains of mice treated with isatin thiosemicarbazone (T.S.) and sacrificed 5 days after initiation of infection was found not to be significantly different from those present in untreated animals(2). Further investigation of the mode of action of thiosemicarbazones in vaccinia infection has demonstrated 1) that serial passage of the virus in treated mice results in a gradual decrease in the amount of virus demonstrable in the brain; 2) that isatin T. S. fails to protect rabbits against vaccinia virus inoculated by the cerebral route; and 3) that aliphatic oxime derivatives of thiosemicarbazone may possess the capacity to protect mice against vaccinia virus. Results obtained from these experiments will be presented in this report.

Methods and materials. The International Health Board (IHD) and the Williamsport strains of virus were used(1,2). Infected mouse brain (IHD strain) or rabbit testicular tissue (Williamsport strain) was ground in a mortar and diluted with Locke's solution containing 5% normal rabbit serum. Harlan Swiss female mice weighing from 15 to 20 g were anesthetized with nembutal and inoculated intracerebrally with appropriate viral dilutions. Rabbits weighing approximately 2 kg, each, were inoculated either intracerebrally or intracisternally. Test materials were fed in a casein-sucrose diet or injected intraperitoneally as a suspension in sterile distilled water.

Results. Two experiments were caried out to determine the effect of serial passage of the Williamsport strain of virus in mice treated intraperitoneally with isatin T.S. (Table I). Animals were treated on the day of viral inoculation and on 2 succeeding days and sacrificed on the 5th day. Brains were removed from 6 mice after each transfer; those found free of bacterial contaminants were pooled and appropriate dilutions prepared. Mice were inoculated for transfer purposes and the viral content of the pool determined by duplicate intradermal titrations in 2 rabbits.

In Exp. 1, mice were treated with 5 mg doses of the compound and passages were

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TABLE I. Effect of Isatin Thiosemicarbazone on Concentration of Vaccinia Virus in the Brain of the Mouse.

Exp.	No. of passage	Conc. (log) of virus in brains of mice sacrificed 5 days after intracer. inoculation of virus: Rabbit titration Treated	Rabbit titration Untreated	Mouse titration Treated	Mouse titration Untreated
1*‡	1	5.1	5.8		
	2	4.3	6.3		
	3	3.4	6.5		
	4	2.8	7.3		
2*§	1	5.5			
	2	4.4			
	3	3.8			
	4	3.4			
	5	2.1			
	6	2.3			
	7	1.4			
3†‖	1	<2.8	<2.5	2.0	<1.5
		3.1	4.7	<1.7	2.9
		4.3	5.3	2.7	2.3
		4.7	6.4	2.1	>4.1
		5.1	6.8	3.1	3.7
		6.1	6.8	>4.0	4.3
Composite titer		4.3	5.2	2.4	3.4

* Brains from 6 mice sacrificed at each passage were pooled before titration. Passages were made with 10^{-3} dilution of brain tissues in Exp. 1 and 10^{-2} in Exp. 2.

† Individual brains were titrated.

‡ Williamsport strain; treatment 5 mg interper. 0,1,2 d.

§ Williamsport strain; treatment 2.5 mg intraper. 0,1,2 d.

‖ IHD strain; treatment 2 mg intraper. 0,1,2 d.

made with the 10^{-3} dilution. After 4 transfers, the viral titer had decreased 2.3 log units or an average of 0.77 log unit for each passage. In contrast, an increase in concentration of virus was observed in brains of untreated control mice. In Exp. 2, a dose of 2.5 mg of thiosemicarbazone was used and transfers were made with the 10^{-2} dilution of infected tissues. After 7 passages, the titer had decreased 4.1 log units or an average of 0.68 units for each transfer.

Since the possibility existed that treatment of mice with isatin T.S. might reduce virulence of vaccinia virus for the mouse but not for the rabbit, a third experiment was carried out in which brains of treated and untreated mice were removed 5 days after viral inoculation and titrated individually in rabbits and in mice. The IHD strain of virus was used in this experiment. The difference between the composite titers of the brains of 6 treated mice was 1.9 log units less when titrated in mice than when titrated in rabbits (Table I). A comparable value of 1.8 log units was obtained for 6 brains from untreated mice. The titer of untreated mouse brains was 0.9 log unit higher than for brains of treated animals when the tissues were titrated in rabbits and 1.0 unit higher when titrated in mice.

Tests in rabbits. In previous experiments it was observed that rabbits treated with thiosemicarbazones were not protected against vaccinia virus injected into the skin(3). Tests were made to determine whether protection could be obtained by similar treatment if the virus were inoculated by cisternal or cerebral routes. It was found that protection was not obtained when isatin T.S. was administered intraperitoneally in 3 doses of approximately 250 mg/kg, each, within 48 hours after viral inoculation.

In contrast, the fatality rate of mice inoculated intracerebally with the same dilutions of the same strain of virus was reduced by about 40% by similar treatment with doses of approximately 90 mg/kg of isatin T.S.

Aliphatic oxime thiosemicarbazones. Thiosemicarbazone derivatives of butanedione, containing oxime or methoxime groups, protect mice against vaccinia virus (Table II). The corresponding semicarbazones are inactive. When fed in a concentration of 0.1%, 1 - phenylethane - 1,2 - dione - 2-oxime-1-T.S. slightly increased the number of survivors in one experiment (Williamsport strain). Pyruvamide T.S. likewise exerts a protective action when fed in a strength of 0.2%. Other materials tested and found to produce little or no protection include acetylacetone di-T.S. (IU), octane - 2,3 - dione - 3 - oxime - 2 - T.S. (WRL), cyclohexan-1,2-dione-phenylhydrazone T.S. (WRL) and butane-2,3-dione-2-oxime-3-S-methyl thiosemicarbazone (WRL). When injected intraperitoneally in doses of 0.3 mg on 0, 1 and 2 days after viral inoculation, butane-2,3-dione-2-methoxime-3-T.S. increased the number of survivors (excess of 4). This compound as well as butane-2,3-dioneoxime T.S., 1-phenylethane-1,2-dione-2-oxime-1-T.S. and acetonylacetone di-T.S.,

TABLE II. Effect of Aliphatic Oxime Thiosemicarbazones and Related Materials on Vaccinal Infection in the Mouse (IHD Strain).

Source*	Compound	Conc. in diet (%)	Proportion of mice surviving infection† Treated	Untreated	Excess of survivors in treated groups
EKC	Butane-2,3-dione oxime T.S.	.1	28/35	6/35	11‡
WRL	Butane-2,3-dione oxime semicarbazone	.1	4/17	4/18	0
"	Butane-2,3-dione, 2-methoxime, 3-T.S.	.015	32/36	13/36	9‡
		.03	34/54	7/53	9‡
"	Butane-2,3-dione, 2-methoxime, 3-semicarbazone	.1	5/18	4/18	1
"	Propane-1,2-dione,1-oxime, 2-T.S.	.1	9/18	5/18	4
"	Octane-2,3-dione, 3-oxime, 2-T.S.	.16	0/18	5/18	−5
"	1-Phenylpropane-1,2-dione-2-oxime-1-T.S.	.16	9/18	4/17	5
"	1-Phenylpropane-1,2-dione-2-methoxime-1-T.S.	.06	8/16	4/18	4
IU	Ethyl pyruvate T.S.	.2	6/18	4/18	2
"	Pyruvamide T.S.	.2	10/16	4/18	6
"	Acetylacetone di-T.S.	.1	1/18	5/18	−4

* Source of compounds: EKC—Dist. Products Industries; WRL—Dr. G. H. Hitchings, Wellcome Research Lab.; IU—Dr. E. Campaigne, Indiana University.

† Approx. equal numbers of mice (6 per group) received 10^{-2}, 10^{-3} and 10^{-4} dilutions of virus intracer.

‡ Composite of 2 or more experiments; avg No. of excess survivors per experiment listed.

in concentrations of 0.001 mg/ml, reduced viral multiplication to a variable degree in tissue cultures containing mouse embryonic tissues.

Minerals. Since butane-2,3-dioneoxime T.S. has been reported to be a chelating agent for manganese (4), tests were made to determine whether this element would counteract the protective capacity of thiosemicarbazones against vaccinia virus. In these experiments, butane-2,3-dioneoxime T.S., butane-2,3-dione-2-methoxime-3-T.S. and isatin T.S. were used. Although, in some experiments, fewer mice survived in groups fed a combination of manganese chloride (.5-1.0%) and thiosemicarbazone than in those treated with thiosemicarbazone alone, the results were irregular and are not interpreted as a reversal of the protective capacity of the thiosemicarbazone. No reduction in the number of survivors was obtained by feeding thiosemicarbazones in combination with copper chloride (0.05%), cobalt nitrate (0.05%), zinc chloride (0.1%) or sodium molybdate (0.015%). Chelating agents, including dimethyl glyoxime, 8-hydroxyquinoline, ethyl xanthate and the sodium salt of tetraacetic acid, also were tested but were found not to influence the resistance of mice to vaccinia virus.

Discussion. The data which have been presented do not explain the mode of action of thiosemicarbazones in vaccinia infection. Isatin T.S. protects mice against virus inoculated intracerebrally but fails to protect rabbits. In the mouse, the concentration of virus which develops in the brain following intracerebral inoculation is somewhat less in treated than in untreated animals. Nevertheless, it would appear that the concentration of virus in the brains of treated mice may reach a level which would not be tolerated by untreated animals. Isatin T.S. must exert either a direct or indirect action on the virus, however, since maintenance of the virus in treated mice results in a gradual diminution in the amount of virus demonstrable in the brain.

It has been demonstrated that a cyclic component is not essential for antiviral activity of thiosemicarbazones. Although the molecular structure of aliphatic oxime derivatives possessing protective capacity would indicate that such compounds could function as chelating agents, evidence has not been obtained that these or other thiosemicarba-

zones act by interfering with mineral metabolism nor that altered mineral metabolism influences resistance of mice to vaccinia virus. The activity of the methoximes, in which metal chelation would be blocked, also points in this direction, although the possibility of *in vivo* demethylation cannot be ruled out. While it is possible to pick out a structural unit, $=N—C—C=NNHCSNH_2$, which is common to most of the active derivatives, a few active thiosemicarbazones outside the scope of this unit have been found, *e.g.*, benzaldehyde T.S., and a few compounds which possess this unit are inactive, *e.g.*, cyclohexanedione phenylhydrazone T.S. Modifications of the sulfur as in the semicarbazones and S-methyl derivatives result in complete inactivation.

The antibiotic, Netropsin, resembles the thiosemicarbazones in that it protects mice inoculated intracerebrally with vaccinia virus but fails to prevent the development of lesions at the site of virus inoculated into the skin of rabbits(5).

Summary. 1. Serial passage of vaccinia virus intracerebrally in mice treated with isatin thiosemicarbazone results in a gradual diminution in the amount of virus demonstrable in the brain. 2. Treatment with isatin thiosemicarbazone fails to protect rabbits inoculated intracerebrally with vaccinia virus. 3. Aliphatic oxime thiosemicarbazones may be as effective as benzene or heterocyclic derivatives as chemoprophylactic agents against vaccinia virus in mice.

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Effect of Diet on Rat Liver Betaine Transmethylase.* (20691)

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The methylation of homocysteine by betaine in the presence of rat liver preparations *in vitro* was first demonstrated by Borsook and Dubnoff(1). The activity of this enzyme system, called betaine transmethylase, has been shown to be reduced when animals are subjected to a deficiency of folic acid(2) or vit. B_{12}(3). The present experiments were designed to investigate further the effects of diet on the enzyme system.

Methods. Weanling Sprague-Dawley rats were used. The basal diet contained alpha protein,† 18 g; cod liver oil, 3 g; Crisco (hydrogenated vegetable fat), 10 g; salt mix (4), 2 g; sucrose, 66.4 g; inositol, 0.1 g; choline chloride, 0.1 g; DL-methionine, 0.43 g; α-tocopherol acetate, 25 mg; thiamine hydrochloride, 0.5 mg; riboflavin, 0.5 mg; folic acid, 0.5 mg; pyridoxine, 0.5 mg; nicotinic acid, 2 mg; calcium pantothenate, 1 mg; 2-methyl-1,4-naphthoquinone, 25 γ; biotin, 5 γ; vitamin B_{12}, 5 γ. The alpha protein was washed exhaustively with hot water, 95% ethyl alcohol, then dried and ground before incorporation into the diets. This basal diet was fed without supplement to some animals.

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† Alpha protein is an isolated soy bean protein obtained from the Glidden Company, Chicago, Ill.