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Action of Acridines on Agents of the Psittacosis-Lymphogranuloma Group.*

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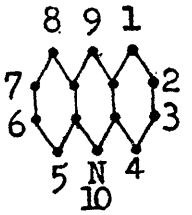
Inhibition of psittacosis infections in mice by trypaflavine (3, 6-Diamino-10-methyl acridinium chloride)† when both drug and



virus were given by the intraperitoneal route has been reported by Mauer.[1] Even when treatment was delayed for 7 days after inoculation of the virus, he observed some therapeutic effect. But in his experiments trypaflavine failed to inhibit cerebral or respiratory infections with psittacosis virus,[1] and Andrewes, King, and van den Ende[2] found that several of the amino acridines and atabrine were without effect on lymphogranuloma venereum virus inoculated into mice by the intracerebral or the respiratory route. Acridines with a nitro substituent at the 3-position inhibit yolk-sac infections with several species of rickettsiae,[3,4] and acriflavine is reported to be rickettsiastatic in chick embryos when the dose is near the toxic level.[3,4] Atabrine, acriflavine, and some other aminoacridines were inactive in mice against respiratory infections with rickettsiae of murine typhus[5] and at concentrations of 1:2,000 were not lethal to this rickettsia *in vitro*. Nitroakridin 3582, or 3 - nitro - 6, 7 - dimethoxy - 9 - (2-hydroxy-3-diethylaminopropylamino) acridine, apparently has an *in vitro* virucidal action or chemoprophylactic effect on influenza virus of type B inoculated into the allantoic sac of chick embryos,[6] but other acridines were ineffective against influenza A in mice.[2] Several aminoacridines and atabrine are reported to inhibit the development of bacteriophage.[7] This paper will describe the action of some of the acridines tried by other investigators, and of a few new derivatives, on the viruses of mouse pneumonitis, cat pneumonitis, meningopneumonitis, and lymphogranuloma venereum.

Materials and Methods. The agent of mouse pneumonitis[8] and the JH strain of the virus of lymphogranuloma venereum[9] were obtained from Dr. Clara Nigg. The cat pneumonitis[10] virus was kindly sent to us by Dr. James A. Baker, and the meningopneumonitis virus,[11] of the strain Cal 10, was originally obtained from Dr. Thomas B. Turner.

Experiments in chick embryos were per-

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† In designating the position of substituents in the acridine nucleus, we have used the following system of numbering throughout:

1 Mauer, G., *Zentralblatt Bakt. Abt. Orig.*, 1938, **142**, 279.

2 Andrewes, C. H., King, H., and van den Ende, M., *J. Path. and Bact.*, 1943, **55**, 173.

3 Smadel, J. E., Snyder, J. C., Hamilton, H. L., Fox, J. P., and Jackson, E. P., *Fed. Proc.*, 1946, II, **5**, 254.

4 Smadel, J. E., Snyder, J. C., Jackson, E. B., Fox, J. P., and Hamilton, H. L., *J. Immunol.*, 1947, **57**, 155.

5 Andrewes, C. H., King, H., and Walker, J., *Brit. J. Pharm. and Chemotherap.*, 1946, **1**, 15.

6 Green, R. H., Rasmussen, H. F., Jr., and Smadel, J. E., *Pub. Health Rep.*, 1946, **61**, 1401.

7 Fitzgerald, R. J., and Babbitt, D., *J. Immunol.*, 1946, **52**, 121.

8 Nigg, C., and Eaton, M. D., *J. Exp. Med.*, 1944, **79**, 497.

9 Rake, G., and Jones, H. P., *J. Exp. Med.*, 1942, **75**, 497.

10 Baker, J. A., *J. Exp. Med.*, 1944, **79**, 159.

11 Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1938, **68**, 147.

formed by inoculating dilutions containing between 3 and 10 LD_{50} of egg-adapted virus, as determined by previous titration, into the yolk sacs of 6-day-old embryos and giving the drug by the same route 2 hours later. In certain experiments the injections of drug were repeated 48 and 96 hours after inoculation of the virus. Control eggs received an equivalent amount of physiological saline solution. The specificity of the deaths and the degree of viral multiplication in the yolk sacs of surviving embryos sacrificed on the 8th day were determined by subinoculation of mice by the intranasal route with yolk sac suspension from individual treated and control eggs. When the yolk sac contained little residual virus, the mice developed pulmonary lesions involving one-third or less of the lung tissue. Heavily infected yolk sacs killed part or all of the mice, and in the survivors sacrificed on the 12th day they were found to have produced a 3-plus or 4-plus pulmonary consolidation.

In experiments in the treatment of respiratory infections, mice were inoculated intranasally with dilutions of 10^{-3} to 10^{-4} of the respective mouse-adapted viruses in lung suspension, the infecting dose being adjusted by previous titration so that animals killed on the 4th day had pulmonary consolidation represented by a lesion score‡ of 20 to 35%, while those killed on the 6th day had lesion scores of 50 to 60%. Drugs were given once daily by the intraperitoneal route beginning 2 hours after the inoculation of the virus. Control mice received virus intranasally and saline intraperitoneally concurrently with the treated animals.

The chemical formula, source, and toxic dose of the 8 acridine compounds used in these studies is presented in Table I. For treatment of chick embryos, compounds W243, W138, and A1150 were ground with a small amount of starch to make suspensions

TABLE I.
Chemical Name and Toxicity of the Acridine Derivatives.

No. or name	Chemical name	Lethal dose mice‖ acute, mg	Lethal dose mice‖ delayed, mg	Lethal dose¶ chick embryos, mg
Acriflavine*	3,6-Diamino-10-methyl acridinium chloride plus 3,6-Diamino acridine HCl	>1.0	4 x 0.5	1.0
Proflavine*	3,6-Diamino acridinium hydrogen sulfate	>1.0	4 x 0.5	1.0
W243†	3-Nitro-6,7-dimethoxy 9-(2-phenyl-4-diethylaminobutylamino) acridine 2 HCl	2.0	4 x 1.0	2 x 0.5
W138†	3-Chloro-7-methoxy 9-(2-phenyl-4-diethylaminobutylamino) acridine 2 HCl	—	4 x 1.0	2 x 1.0
W1889† §	3-Nitro-6,7-dimethoxy 9-(2-hydroxy-3-diethylaminopropylamino) acridine 2 HCl $2H_2O$	5.0	4 x 1.0	2 x 0.5
W10†	3-Chloro-7-methoxy 9-(2-hydroxy-3-diethylaminopropylamino) acridine 2 HCl	5.0	4 x 1.0	2 x 1.0
Atabrine	3-Chloro-7-methoxy 9-(1-methyl-4-diethylaminobutylamino) acridine 2 HCl	2.5	4 x 1.5	2 x 1.0
A1150‡	3-Nitro-9-aminoacridine HCl	1.0	4 x 0.5	2 x 0.5

* Purchased from National Aniline Division, Allied Chemical and Dye Co., Inc.
† Given by Dr. M. L. Tainter, Sterling-Winthrop Research Institute.
‡ Given by Dr. D. L. Tabern, Abbott Laboratories.
§ Same formula as Nitroakridin 3582. See references 3 and 6.
‖ Weight 15-18 g. Delayed lethal dose represents amount of drug given on 4 successive days.
¶ Two doses given 48 hours apart.

‡ The method of recording pulmonary lesion scores was originally used by Horsfall.[12] A lesion score of 100% represents death with complete pulmonary consolidation. In surviving mice, 80% represents, on the average, 4+ consolidation; 60%, 3+; 40%, 2+; and 20%, 1+.

TABLE II.
Effect of Acridines on Yolk Sac Infections with the Cat Pneumonitis Virus in Chick Embryos.

Drug	Dosage mg/egg	Dosage No.	Mortality ratios and degree of infection on 8th day* Treated egg	Control eggs	% reduction of Mortality	% reduction of Heavy infection
Acriflavine	.3	2	0/11 (1)†	15/16 (1)†	—*100*	—*91*
	.3	1	0/9 (2)		—*100*	—*78*
	.2	1	0/6 (4)	8/8	—*100*	—33
	.1	1	8/9 (1)		— 11	0
Proflavine	.3	2	4/5 (1)	6/7 (1)	— 7	0
W243	.3	3	1/14 (2)	15/18	— *91*	—*74*
	.3	1	0/8	12/12	—*100*	—*100*
	.1	1	3/4 (1)		— 25	0
W138	.3	2	7/7	3/4	0	0
W1889	.3	1	0/8		—*100*	—*100*
	.2	1	0/9 (2)	8/8	—*100*	— *78*
	.1	1	4/8 (1)		— *50*	— 38
Atabrine	2.0	1	8/8	8/8	0	0
	.5	3	6/9 (2)	6/7	— 22	0
A1150	.2	2	8/20 (10)	17/18	— *58*	— 4

* 3 to 10 LD_{50} of virus in each experiment.
†Figures in parentheses represent number of surviving eggs which were found to be heavily infected by subinoculation to mice and production of pulmonary lesions involving, on the average, over half of the lungs. Lesion scores in mice inoculated from remainder of living eggs were less than 30%.

in physiological saline which were then autoclaved at 10 lb pressure for 10 minutes. Solutions of the remaining drugs were sterilized by filtration through bacteria-retaining fritted glass discs. When used in mice the solutions or suspensions were not sterilized. In the table the lethal dose is given as the least amount which killed more than half of the mice or chick embryos. The delayed lethal dose for mice is given as the daily dose in milligrams injected intraperitoneally on 4 successive days. In chick embryos, 2 doses separated by an interval of 48 hours were inoculated into the yolk sac.

Results in chick embryos infected with the virus of cat pneumonitis. Seven acridine derivatives were tested in chick embryos against the virus of cat pneumonitis as shown in Table II. The results are evaluated in terms of mortality of the embryos by the 8th day after inoculation, at which time most of the controls were dead. In the 4th and 5th columns the figures in parentheses represent the number of additional eggs in each group which survived with heavy infection, as determined by the mouse test. These probably would have died before the time of hatching. The remainder of the surviving chick embryos had little residual virus at 8 days or none detectable by subinoculation of mice.

The last 2 columns of the table show the percentage reduction of mortality in the treated eggs as compared with the controls, and the percentage reduction in the incidence of heavy infection. The formula 100 (-1+T/C) is used; T represents the percent mortality in the treated eggs, and C the percent mortality in the controls. For estimating the percentage reduction in heavy infections, the figures in parentheses (columns 4 and 5) were added to the numerator of each fraction and the calculations were again carried out with the formula given above. Reductions of 40% or over are italicized, and are considered to be significant.

Acriflavine and the 2 nitroacridines, W243 and W1889, were effective in single doses of 0.2 to 0.3 mg, or 1/3 to 1/5 of the lethal dose for chick embryos. With the possible exception of W1889, no appreciable effect was

TABLE III.
Effect of Acridines on Respiratory Infection with Cat Pneumonitis Virus in Mice.

Drug	Daily drug dosage mg/mouse	Daily drug dosage No.	Mice No.	Mice Day killed	Pulmonary lesion scores in % Treated mice*	Pulmonary lesion scores in % Controls	%† reduction in pulmonary consolidation
Acriflavine	0.2	4	19	4	21	37	*—43*
		5	19	6	52	57	— 9
Proflavine	"	4	18	4	34	37	— 8
		5	19	6	57	57	0
W243	0.4	4	39	4	9.5	32	*—70*
		5	23	6	27	56	*—52*
W138	0.5	4	16	4	36	42	—14
		5	8	6	60	67	—10
W1889	0.4	4	12	4	2.5	24	*—90*
		5	30	6	5.0	56	*—91*
W10	0.5	5	22	6	44	48	— 8
A1150	0.2	4	26	4	21	23	— 9
		5	12	6	47	50	— 6
Penicillin G	7.9‡	4	18	4	8.9	32	*—72*

* Dilutions of virus 2 × 10^{-3} to 5 × 10^{-4} inoculated intranasally.
† 100(—1 + T/C).
‡ 1520 units of penicillin G per mg or 12,000 units per day given in 3 doses daily.

found with doses of 0.1 mg of these substances. In other preliminary experiments, some inhibitory effects were observed with the 3 drugs even when treatment was delayed for 24 or 48 hours after inoculation of the virus. The 3-nitro-9-aminoacridine, A1150, which differs from the others in having no side chain and no methoxy groups (see Table I) caused some reduction in the mortality, but was much less inhibitory than the other 3 drugs when given in equivalent dosage. Proflavine, in contrast to acriflavine, failed to influence the mortality of embryos infected with the cat pneumonitis virus under the conditions of these experiments. A slight inhibitory effect of proflavine on virus growth was demonstrable when the chick embryos were sacrificed 5 days after inoculation and the yolk sacs tested in mice. (The results of this test are not included in Table II.) The two acridines, W138 and atabrine, differ from W243 and W1889, respectively, by the presence of a single methoxy group and the substitution of a chlorine atom for the 3-nitro group. As may be seen in Table II, these 2 chloroacridines had no effect on the virus of cat pneumonitis in chick embryos when given in amounts considerably larger than the therapeutic dose of the corresponding nitroacridines.

Results in mice with cat pneumonitis virus. Mice inoculated by the intranasal route with the virus of feline pneumonitis were given daily doses of the acridine compounds in amounts representing ½ to ⅔ of the toxic dose. The results are presented in Table III. The 3-nitroacridines (W243 and W1889) which were most effective in chick embryos also caused a significant reduction in the pulmonary consolidation of the mice, while the closely related 3-chloroacridines (W138 and W10) were inactive. Acriflavine, proflavine, and 3-nitro-9-aminoacridine (A1150) were about twice as toxic for mice as the other drugs and were, therefore, used in half the amount. The result with acriflavine in mice sacrificed 4 days after inoculation was of borderline significance, and the other compounds had no effect at the maximum tolerated dosage.

For comparison, a group of mice infected with the same virus received 12,000 units of penicillin per day divided into 3 doses of 4,000 units each and given at intervals of 7, 7, and 10 hours. The resulting inhibition of pulmonary consolidation in the mice was the same or slightly less than that caused by the nitroacridines. It should be noted that the acute lethal dose of the crystalline penicillin G used in these experiments was greater than 50,000 units.

Mouse pneumonitis, lymphogranuloma ven-

TABLE IV.
Action of Acridines on the Viruses of Mouse Pneumonitis, Lymphogranuloma Venereum, and Meningopneumonitis in Mice and Chick Embryos.

Drug	Virus (10 LD_{50})	Results in chick embryos* Dose,* mg	No. tested	% change	Results in mice† Dose, mg	No. tested	% change
Acriflavine	Mouse Pn.	0.3	16	— 35	0.2	40	+10
	LGV	"	6	*—100*	"	24	*—40*
	Meningopn.	"	19	*— 86*	"	24	—14
W243	Mouse Pn.	"	20	— 16	0.4	31	+19
	LGV	0.2	13	*— 54*	"	28	+ 6
	Meningopn.	"	16	*— 67*	"	27	— 6
W1889	Mouse Pn.	"	10	*— 70*	0.3	24	—18
	LGV	"	9	*— 87*	"	24	*—46*
	Meningopn.	"	9	*— 89*	"	24	*—40*

* Drug given to chick embryos 2 and 48 hours after virus. Results expressed as reduction of incidence of heavy yolk sac infection in embryos harvested at 8 days when 75 to 100% of control embryos were heavily infected (see Table II).

† Mice received drug daily as in Table III. Results averaged for groups killed at 4 and 6 days, expressed as percentage reduction or increase in lesion scores of treated mice as compared with controls.

ereum, and meningopneumonitis in chick embryos and mice. A summary of the results of preliminary experiments with 3 other viruses of the group is presented in Table IV. The drugs most effective against the cat pneumonitis virus, acriflavine and the nitroacridines W243 and W1889, were used in amounts and dosage schedules comparable to those listed in Tables II and III. In chick embryos, all 3 drugs definitely inhibited the growth of the viruses of lymphogranuloma venereum and meningopneumonitis, the effects being similar to those obtained with the agent of feline pneumonitis when expressed as reduction in incidence of heavy infection (see last column of Table II). Against the virus of mouse pneumonitis acriflavine gave results which were just below the level of significance and W243 showed no therapeutic activity. In other experiments not included in Table IV, retarded growth of this virus in embryos treated with acriflavine or W243 was observed when the yolk sacs were harvested at 5 days. The other nitroacridine (W1889) caused more definite inhibition of the growth of the mouse pneumonitis virus in chick embryos.

In mice the inhibition of respiratory infections with the 3 viruses mentioned above was less striking than that occurring in infections with the cat pneumonitis virus. None of the drugs produced appreciable reduction in the pulmonary consolidation resulting from the mouse pneumonitis virus. With one nitroacridine, W1889, results of borderline significance were obtained in tests against the viruses of lymphogranuloma venereum and meningopneumonitis, but the other nitroacridine, W243, had no effect on these 2 viruses in the lungs of mice. Acriflavine showed questionable activity against pulmonary infections with the virus of lymphogranuloma venereum and none against the agent of meningopneumonitis.

Discussion. The results of these experiments suggest that replacement of the NO_2 group at the 3-position on the acridine nucleus by Cl results in loss, or marked diminution, of the inhibitory action against the virus of feline pneumonitis. Since the relatively simple compound 3-nitro-9-aminoacridine had some inhibitory action in chick embryos, it seems that the other substituents such as the 6- or 7- methoxy groups and the 9-dialkylaminoalkylamino side chain are not absolutely essential to the antiviral activity. The possible role of the side chain at the 9- position must be investigated further, because the compounds with this substituent seemed to be somewhat more active on weight basis than the simple 3-nitro-9-aminoacridine. Also, the preliminary results in Table IV suggest a slightly wider range of activity for the compound with the 9-(2-hydroxy-3-diethylaminopropylamino) substituent (W1889) as compared with the 9-(2-phenyl-4-diethylamino-

butylamino) group (W243).

It remains to be seen whether acriflavine acts in the same manner as the nitroacridines. The active constituent of acriflavine seems to be the 10-methyl acridinium chloride (see Mauer[1]). Proflavine, which is the sulphuric acid salt without the 10-methyl group, was much less inhibitory for the feline pneumonitis agent in chick embryos. Its activity against other viruses of the group is under investigation.

The relative resistance of the mouse pneumonitis virus to acriflavine and to one of the nitroacridines is surprising in view of the high sensitivity of this agent to penicillin[13,14] and sulfonamides.[15] The feline pneumonitis and meningopneumonitis viruses which are, on the other hand, relatively resistant to penicillin and are not affected in mice or chick embryos by sulfonamides are quite readily inhibited in chick embryos by acriflavine and the nitroacridines. The observed differences in the chemotherapeutic spectrum for penicillin, sulfonamides, and acridines suggest interesting variations in the enzymatic or metabolic constitution of the agents of the psittacosis-lymphogranuloma group. The 3 classes of substances probably act on 3 different constituents of the viruses under consideration.

The results in mice tended to parallel those in chick embryos, but on a lower scale of activity so that less conclusive results were obtained. Effective inhibition of respiratory infections was observed only with the 2 nitroacridines against the cat pneumonitis agent, but these 2 substances were, on a weight basis, more effective than penicillin against this virus. Because of the high toxicity of acridines and their failure to show general activity against the psittacosis-lymphogranuloma group in mice, no claim for their therapeutic usefulness can be made at the present time.

Summary. Acriflavine; 3-nitro-6, 7-dimethoxy 9 - (2-phenyl-4-diethylaminobutylamino) acridine; and 3-nitro-6, 7-dimethoxy 9 - (2-hydroxy - 3-diethylaminopropylamino) acridine inhibited yolk sac infections of chick embryos with the agents of feline pneumonitis, lymphogranuloma venereum, and meningopneumonitis. The first two compounds were less active against the virus of mouse pneumonitis, but the last-named inhibited this agent in chick embryos.

Proflavine, atabrine, and drugs closely related to the above-mentioned nitroacridines, except for substitution of Cl for NO_2, had no significant inhibitory action. 3-nitro-9-aminoacridine was intermediate in its effect.

Respiratory infections in mice caused by the agent of feline pneumonitis were retarded by the two nitroacridines, but these drugs showed slight or no effect in mice against intranasal infection with the agents of mouse pneumonitis, lymphogranuloma venereum, and meningopneumonitis.

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14 Eaton, M. D., Dozois, T. F., van Allen, A., Parish, V. L., and Schwalm, S., *J. Immunol.*, in press.

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16012

Effect of Egg Yolk on Release of Antigen from Rickettsiae.

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The existence of a soluble antigen which is released on treatment of certain rickettsiae with ether has been well established with respect to *Rickettsia prowazeki*,[1] *R. rickettsii*,[2] and *R. akari*.[3,4] That this antigen, in the case

1 Topping, N. H., and Shear, M. J., *Pub. Health Rep.*, 1944, **59**, 1671.