cold environment is to lower the resistance of mice to the lethal action of X-rays.

Subjection to a 10° or 30°C environment for 2 weeks prior to irradiation favors survival of irradiated mice maintained in those environments.

Mice kept in a 30° environment that die following irradiation frequently show fatty changes in the liver.

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17238. Effect of Nitrocompounds on Viruses of the Psittacosis-Lymphogranuloma Group.

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Because of the observation that nitroacridines showed a marked inhibition of agents of the psittacosis-lymphogranuloma group[1,2] while chloro-substituted and certain other acridines were ineffective,[1] it seemed desirable to investigate the activity of nitrocompounds other than acridines. Substances containing a benzene or furan ring were selected for investigation because certain structural features in nitroacridine are also present in the latter, simpler compounds. While these studies were in progress other publications have described activity of two compounds containing a nitro-phenyl group against viruses of the psittacosis group or rickettsiae. One of these is chloramphenicol (chloromycetin)[3,4] and the other a nitro analogue of D.D.T., 1,1, 1-trichloro-2, 2-bis (para-nitrophenyl) ethane which was found to show some effectiveness against murine typhus in mice.[5]

Material and methods. The source of the strains of the viruses of lymphogranuloma venereum, meningopneumonitis, cat pneumonitis, and mouse pneumonitis is given in a previous publication.[1] Experiments in mice 16 to 18 grams in weight were done with mouse lung passage of these viruses inoculated by the intranasal route. Titrations were done and the infecting dose adjusted so that control mice in each experiment when killed on the 6th day had pulmonary lesions giving a score between 30 and 60.† Drugs were given by the intraperitoneal route as single daily doses starting 2 hours after the virus. Control mice received saline intraperitoneally.

The experiments in chick embryos were done with yolk sac passages of the four viruses inoculated either into the yolk sac or the allantoic sac. The technic of the experiments using inoculation into the yolk sac with about 10 LD_{50} was identical with that previously described.[1] In the other series of experiments 10 to 100 ID_{50} (50% infectious doses) were inoculated into the allantoic sac of 11-day-old chick embryos and a single dose of drug inoculated after the virus either into the allantoic sac or into the yolk sac. Control embryos received saline. The embryos were sacrificed on the 5th day after inoculation and the degree of viral multiplication in the allantoic sac determined by inoculating allantoic fluids from individual treated and control eggs into mice by the intranasal route. Fluids

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3 Smadel, J. E., and Jackson, E. B., *Science*, 1947, **106**, 418.

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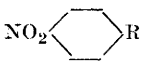
† A lesion score of 100 represents death with complete pulmonary consolidation. In surviving mice 80 represents on the average 4+ consolidation; 60, 3+; 40, 2+; and 20, 1+.

TABLE I.
Effect of Substituted Nitrobenzenes on Viral Respiratory Infections in Mice.

Virus	Substituent R*	Daily dose, mg	No. of mice	Lesion score		Lung wt (g)	
				T/C†	% change‡	T/C†	% change‡
Cat pneumonitis	COONa	10	43	29/41	—29	0.248/0.288	—14
	$CONH_2$	5	18	24/40	—40	0.192/0.247	—23
	$C(NH)NH_2$	2	40	30/40	—25	0.284/0.313	— 9
	SO_2NH_2	4	23	25/52	—52	0.221/0.305	—27
	(Sulfanilamide)	10	14	42/46	—11	0.290/0.293	— 1
	Chloramphenicol	2	16	36/38	— 5	0.402/0.340	+18
	"	10	16	47/73	—36	0.374/0.460	—19
Mouse pneumonitis	COONa	10	25	39/34	+14	0.295/0.269	+10
	$CONH_2$	5	20	35/31	+13	0.220/0.255	—14
	$C(NH)NH_2$	2	53	33/38	—13	0.312/0.305	+ 2
	SO_2NH_2	4	21	18/52	—65	0.168/0.260	—35
	(Sulfanilamide)	10	13	20/57	—65	0.173/0.273	—37
	Chloramphenicol	2	16	41/46	—11	0.282/0.376	—24
Lymphogranuloma venereum	COONa	7.5	27	38/39	— 3	0.261/0.266	— 2
	$CONH_2$	5	16	36/40	—10	0.244/0.280	—14
	Chloramphenicol	2	16	23/41	—41	0.204/0.345	—41
Meningopneumonitis	COONa	7.5	26	33/35	— 6	0.255/0.271	— 6
	$CONH_2$	5	33	26/32	—19	0.312/0.323	— 3
	SO_2NH_2	3	31	39/46	—16	0.348/0.379	— 8
	Chloramphenicol	2	16	37/51	—28	0.387/0.423	— 9
	"	5	16	21/42	—50	0.295/0.456	—35

* Substituents in para position to nitro group:



R; for chloramphenicol R = $CH(OH)CH(NH_2COCHCl_2)CH_2OH$

† T = Treated mice; C = Control mice.
‡ % change = 100 (—1 + T/C).

were considered to be significantly infected when they produced average lesion scores of 20 or over in the group of 3 mice subinoculated from each embryo.

Drugs were injected by the intraperitoneal route in mice without heating or filtration, insoluble drugs being ground with a small amount of starch and suspended in saline. For inoculation of chick embryos the drugs were sterilized in saline suspension or solution by heating in a boiling water bath for 20 minutes. One nitrofuran which was soluble but unstable at 100°C was sterilized by filtration through a fritted glass filter. Dosage up to one-half or one-third of the maximum tolerated dose was used in these experiments.

Results in mice. These were expressed as reduction of the observed gross pulmonary consolidation and the weight of the lungs of the treated mice as compared with the controls. Further details on the measurement of the lung weights and their relation to the degree of consolidation will be found in another publication.[6] In most instances two or three experiments were done with the same drug and when the effects in each experiment were similar the results were averaged. A summary of the findings in mice with compounds containing a nitrophenyl group is presented in Table I. With four compounds, sodium p-nitrobenzoate, p-nitrobenzamide, p-nitrobenzamidine, and p-nitrobenzene sulfonamide, evidence of inhibition of the cat pneumonitis virus was obtained. The most significant effects were those with the two acid amides. With the exception of p-nitrobenzene sulfonamide these substances had no effect against the agent of mouse pneumonitis. Since the latter virus is sensitive to the action of sulfanilamide the positive result with p-

[6] Huang, C., and Eaton, M. D., *J. Bact.*, 1949, in press.

TABLE II.
Effect of Nitrofurans on Viral Respiratory Infections in Mice.

Virus		Substituent R*	Daily dose, mg	No. of mice	Lesion score T/C	Lesion score % change	Lung wt (g) T/C	Lung wt (g) % change
Cat pneumonitis	(I)	$CH{=}N{-}N(H){-}C(=O){-}C(=O){-}NH_2$	3	22	31/48	—35	0.228/0.353	—35
	(II)	$CH{=}N{-}N(CH_2{-}CH_2OH){-}CONH_2$ (H above N)	2	15	20/45	—56	0.236/0.393	—40
Mouse pneumonitis	(I)	$CH{=}N{-}N(H){-}C(=O){-}C(=O){-}NH_2$	3	27	26/56	—54	0.201/0.312	—36
Lymphogranuloma venereum	(I)	$C{=}N{-}N(H){-}C(=O){-}C(=O){-}NH_2$	3	27	6/51	—88	0.178/0.290	—39
	(IV)	$CH(CO_2CH_3)_2$	2	26	18/37	—51	0.217/0.301	—28
Meningo-pneumonitis	(I)	$C{=}N{-}N(H){-}C(=O){-}C(=O){-}NH_2$	3	36	11/36	—70	0.211/0.301	—30
			2	16	18/51	—41	0.333/0.423	—21
	(III)	$C{=}N{-}N(H){-}C(=O){-}NH_2$	3	31	21/36	—42	0.238/0.340	—30
	(II)	$C{=}N{-}NCONH_2$ with CH_2CH_2OH on N	2	20	26/40	—35	0.288/0.379	—24
	(IV)	$CH(CO_2CH_3)_2$	2	20	41/49	—16	0.372/0.418	—11

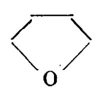
* All are derivatives of 5-nitro-2-furaldehyde NO_2  —R where R represents the aldehyde group combined as semioxamazone (I) 2-β-hydroxyethyl-semicarbazone (II), semicarbazone (III), or diacetate (IV).

TABLE III.
Effect of Nitro Compounds on Allantoic Infections with Cat Pneumonitis Virus in Chick Embryos.

Drugs			Degree of infection as indicated by subinoculation of allantoic fluid into mice†			
Name	Dose, mg	Route	LS<20	LS20-50	LS>50	Average L.S.
Sodium p-nitrobenzoate	10	A	11	6	2	27
p-Nitrobenzamide	10	YS	4	3	1	25
Saline		A	3	9	16	78
p-Nitrobenzene sulfonamide	1	A	6	8	1	22
(Sulfanilamide)	1	A	2	10	3	37
Saline		A	0	10	5	47
NF II‡	0.5	YS	6	3	1	24
Chloramphenicol	1	YS	6	2	0	7
Saline		A	4	6	2	31
(Penicillin G)	0.3	YS	14	3	3	18
Saline		YS	3	3	9	65

* A = Allantoic; YS = Yolk sac.
† Eggs are divided into three groups; number not significantly infected, lesion score (LS)<20 in mice; number moderately infected, LS20-50; number with heavy infection, LS>50. Average lesion score for entire group of eggs is given in last column.
‡ NF II = 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone.

nitrobenzene sulfonamide may be attributed to reduction of the nitro group to an amino group in the body. The data from a comparable experiment with sulfanilamide included in Table I show that the effect was very similar in degree. It will be seen, however, that sulfanilamide had little effect on the cat pneumonitis virus while the various nitro-compounds showed slight or moderate activity. With p-nitrobenzamide and p-nitrobenzene sulfonamide, the reduction of pulmonary lesions in mice infected with the agents of lymphogranuloma venereum and meningopneumonitis were very slight and in most cases probably not significant.

Chloramphenicol‡ at a dosage of 2.0 mg/mouse daily had no significant effect against the virus of cat pneumonitis, and only a slight effect against the virus of mouse pneumonitis. At this dosage level a definite inhibitory effect was obtained against the virus of lymphogranuloma venereum but the activity against the agent of meningopneumonitis was of doubtful significance. By increasing the dose definite effects were obtained against the cat pneumonitis virus with 10 mg per day and against the meningopneumonitis virus with 5 mg.

‡ Kindly furnished by Dr. F. Stimpert, Parke Davis & Company.

Similar experiments in mice were done with four derivatives of 5-nitro-2 furaldehyde§ using daily doses of 2 to 3 mg per mouse, which was close to the maximum amount tolerated over a period of one week. The mortality from toxic effects of the drugs ranged from 10 to 30% of the treated mice in most of the experiments with these substances. From the results presented in Table II, it will be seen that these nitrofurans had definite inhibitory activity against the agents of mouse pneumonitis, cat pneumonitis, lymphogranuloma venereum, and meningopneumonitis. The most active compound under the conditions of these experiments was the semioxamazone, while the diacetate was found to be the least effective. With the former compound at a dosage of 3 mg marked inhibition of the pulmonary lesions produced by the virus of lymphogranuloma, and the meningopneumonitis virus were observed. When the dosage was reduced to 2 mg toxicity of the drug was not evident in the 6-day period of the experiment, but the effect on the pulmonary lesions produced by the meningopneumonitis virus was not as great.

§ 5-nitrofuraldehyde diacetate was purchased from the Eastman Kodak Co. The other three compounds were given by Dr. Mary F. Paul of the Eaton Laboratories, Inc.

TABLE IV.
Effect of Nitrocompounds on Allantoic Infections with Meningopneumonitis Virus in Chick Embryos.

Drugs			Degree of infection as indicated by subinoculation of allantoic fluid into mice			
Name	Dose, mg	Route	LS<20	LS20-50	LS>50	Average L.S.
NF I*	1.0	A	10	12	7	37
Saline		A	2	1	27	83
NF II†	1.0	YS	4	1	1	15
Saline		A	1	2	3	59
Nitroakridin 3582	0.3	A	14	0	0	1.5
Saline		A	1	3	14	74

* NF I = 5-nitro-2-furaldehyde-semioxamazone autoclaved in dry state.
† Filtered through fritted glass (see Tables II and III for name and formula).

Results in chick embryos. Evidence for inhibition of the multiplication of the viruses by nitro-compounds was obtained from experiments in chick embryos. Since the effectiveness of p-nitrobenzoic acid or its amide and p-nitrobenzene sulfonamide seemed to be limited largely to the agent of cat pneumonitis only the experiments in chick embryos with this virus are presented in Table III. Eleven-day-old embryos were infected with a 10^{-3} dilution of yolk sac suspension given by the allantoic route. One hour later the drug was inoculated either by the same route or into the yolk sac. Embryos seldom died as the result of infection but were sacrificed on the 5th day after inoculation and the amount of virus in the allantoic fluid measured by the pulmonary lesion scores resulting from subinoculation intranasally of these fluids into mice which were then sacrificed on the 14th day. As shown in Table III, the allantoic fluids from the majority of the embryos treated with the p-nitrophenyl compounds contained insufficient virus to produce lesion scores of 20 or more in mice while the fluids from many of the control eggs produced lesion scores over 50. The average of the lesion scores from the individual eggs in each group is shown in the last column. The effects were considered significant when the average from the treated eggs was one-half to one-third of the average lesion scores produced by fluids from the control eggs. Chloramphenicol was found to be much more effective against allantoic infections with this virus in chick embryos than it was in mice. The results with sulfanilamide and penicillin are included for comparison. Sulfanilamide at a dosage of 1 mg, comparable to p-nitrobenzene sulfonamide, had no significant effect. The effect produced by a single dose of 0.3 mg (500 units) of penicillin G was equivalent to or only slightly greater than the effect of the nitro-compounds.

The results of similar experiments with nitrofurans and the agent of meningopneumonitis are presented in Table IV. A number of experiments with these compounds sterilized by heating suspensions in saline or starch-saline at 100° for 20 minutes were negative, probably because of heat lability of the drug.‖ Several experiments were then done with the semioxamazone which was sterilized by heating in the dry state, but the results were not as good as would be expected from the experiments in mice. Since 5-nitro-2 furaldehyde-2-β-hydroxyethyl semicarbazone is soluble in water to the extent of slightly more than 1 mg per cubic centimeter at room temperature, solutions of this substance were filtered and 1 cc inoculated into the yolk sac. In this experiment a definite inhibition of growth of the meningopneumonitis virus in the allantoic sac was observed. In a similar experiment with the cat pneumonitis virus (see Table III) using a dose of 0.5 mg of this same substance no effect was obtained. The results of an experiment with nitroakridin 3582, (3 nitro- 6,7-dimethoxy-9-(2-hydroxy-3-diethyl amino propylamino) acridine, are also included in Table IV. It is obvious that this compound produced a much more marked inhibition than the nitrofurans in the allantoic sac but from previous investi-

‖ Mary F. Paul, personal communication.

TABLE V.
Effect of Nitrocompounds on Yolk Sac Infections in Chick Embryos.

Virus	Drug*	Dose	Degree of infection†				Mortality ratio
			LS<20	LS20-50	LS>50	D	
Mouse pneumonitis	PNB	9 mg	2	2	0	5	5/9
	Saline		3	1	1	8	8/13
Cat pneumonitis	PNB	9 mg	8	0	1	2	2/11
	Saline		5	0	0	10	10/15
	NF II	0.5 mg	7	0	3	0	0/10
	Saline		0	0	2	7	7/9
Meningo-pneumonitis	PNBa	10 mg	0	1	1	4	4/6
	Saline		0	0	4	4	4/8
	NF II	0.5 mg	4	2	0	0	0/6
	Saline		0	1	1	9	9/11

* PNB = p-Nitrobenzoic acid-sodium salt.
PNBa = p-Nitrobenzoic acid-amide.
NF II = 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone sterilized by filtration.
† In eggs surviving to 8 days. D represents number of embryos dead by 8 days.

gations,[1] it seemed to be less effective than the nitrofurans against the meningopneumonitis virus in the lungs of mice.

The results of experiments in which both drug and virus were inoculated into the yolk sacs of chick embryos are presented in Table V. Sodium p-nitrobenzoate in large doses had no effect on the multiplication of the mouse pneumonitis virus but definite evidence of inhibition of the virus of cat pneumonitis was obtained. In one experiment p-nitrobenzamide had no effect on the agent of meningopneumonitis. The compound 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone inhibited the growth of both the cat pneumonitis and meningopneumonitis virus in the yolk sac. While most of the control eggs in these two experiments were either dead or heavily infected by the 8th day after inoculation, the majority of the treated eggs survived with insignificant amounts of virus, although a few had moderate to heavy infections. These results with the three viruses and two types of compound parallel quite closely the observations with mice as reported above.

Negative results. The following additional substances containing a nitrophenyl group were screened against the virus of meningopneumonitis in mice and in some cases against other viruses in mice and chick embryos and found to have no effect: p-nitrobenzene sulfonanilide, m-nitrobenzene sulfonamide, sodium p-nitrobenzene sulfonate, p-nitroacetanilide, 3-nitro-4-acet-toluide, N(5-nitro-2-furylidene)-1-aminohydantoin.

Discussion. These observations and the results reported by other investigators suggest that a certain chemical structure containing a nitro substituent confers on several substances activity against viruses of the psittacosis-lymphogranuloma group. The only chemical grouping common to most of the active nitro compounds so far found is the ring-system of carbon atoms with conjugated double bonds thru which the nitro group is joined to various chemical complexes of rather wide diversity.

It is likely that the nature of the substituent on the benzene ring in the para position to the nitro group plays an important part in determining chemotherapeutic and pharmacologic properties. An analogy with the sulfonamides exists in that the substituent on the sulfonic acid group of the latter compounds increases or decreases their activity. Although some of the compounds listed in the present work resemble p-aminobenzoic acid, others such as the nitrofurans have a structure which is quite different from PABA. Since PABA itself has been found to be inactive against agents of the psittacosis-lymphogranuloma group[6,7] the effects of p-nitrobenzoic acid and its derivatives cannot be at-

tributed to reduction of the NO_2 group to NH_2 *in vivo*. The possible effect of this reduction in other compounds has not to our knowledge been investigated.

From a consideration of the spectrum of antiviral activity of these substances it is evident that the nitro-compounds as a group, including nitroacridines, have a somewhat similar range of activity while differences from sulfonamides may be found. The agent of mouse pneumonitis which is highly sensitive to sulfonamides, has a relatively low sensitivity to nitroacridine[1] and is not inhibited significantly by derivatives of p-nitrobenzene with the exception of chloramphenicol. The cat pneumonitis and meningopneumonitis viruses are resistant to sulfonamides but sensitive to the action of nitroacridines and to most of the other nitro compounds in the case of the former virus but only to nitrofurans and chloramphenicol in the case of meningopneumonitis.

Under the conditions of these experiments the nitrofurans seemed to have a wider range of activity against viral respiratory infections in mice than did the other nitro compounds. The results with chloramphenicol are in agreement with the report of Smadel and Jackson[8] who demonstrated activity of this substance against the agents of lymphogranuloma venereum and psittacosis in chick embryos and against psittacosis in mice inoculated by the intraperitoneal route but were unable to find significant activity against these viruses in mice inoculated by the intracerebral route. From the results presented in Tables I and II it may be seen that chloramphenicol seemed to be less active against the agent of cat pneumonitis than were some of the other nitro compounds. On the other hand, chloramphenicol was more active against the agents of lymphogranuloma venereum and meningopneumonitis than were any of the other nitrophenyl compounds, but was somewhat less active than the nitrofurans.

While the observations reported here reveal interesting relationships between chemical structure and activity against the viruses of the psittacosis-lymphogranuloma group, it is doubtful that the chemotherapeutic index of any of the substances tested is favorable enough to justify human use. Although sodium p-nitrobenzoate is of low toxicity in mice it has a relatively low activity which is limited almost entirely to the agent of feline pneumonitis. Nitrosulfonamides have been used in human beings for other purposes,[9] but their activity against the psittacosis-lymphogranuloma group is also limited. The nitrofurans have a relatively high degree of activity against these viruses but they have a definite toxic action and have been shown to produce delayed lethal effects in guinea pigs several days after discontinuance of administration.[10] Chloramphenicol is probably among the least toxic of the substances tested but its activity against the agents of the psittacosis-lymphogranuloma group does no seem to be greater than that of penicillin or the sulfonamides.[8]

Summary. Several substituted nitrobenzenes including chloramphenicol (chloromycetin) show inhibitory activity against the viruses of meningopneumonitis, lymphogranuloma venereum, cat pneumonitis, and mouse pneumonitis. Several nitrofurans were also shown to inhibit the growth of these viruses. The chemotherapeutic activity of these various substances is compared and the relation of chemical constitution to activity discussed. Although chloramphenicol is probably the least toxic of the substances tested, it did not show striking superiority to other nitro-compounds in activity against viruses of the psittacosis-lymphogranuloma group under the conditions of these experiments.

[7] Hamilton, H. L., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 220.

[8] Smadel, J. E., and Jackson, E. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 478.

[9] Major, R. H., *J. Lab. and Clin. Med.*, 1946, **31**, 219.

[10] Wolinsky, E., Wetzel, V., and Steenken, W. J., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 483.

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