mice by the feeding of a diet of the "synthetic" type might be attributed to the greater nutritional adequacy of the synthetic diet as compared with the commercial pellet, or to the greater fat content of diet No. 226. Further experiments are needed to answer this question. Fatty livers such as shown by the yellow mice fed diet No. 226 have been reported in mice of the A and C_3H strains by Fenton and Mershon(6).

The occurrence of two liver tumors seems worthy of mention although no data are available on the incidence of liver tumors in these yellow sublines. The existence of such tumors in animals only 6 months of age merits further attention.

Summary and conclusions. 1. The observation has been confirmed that yellow mice on continued inbreeding no longer become obese if maintained under the usual laboratory conditions. 2. Feeding a diet of the "synthetic" type made it possible to induce obesity in the yellow mice but not in their non-yellow littermates. 3. The obese yellow mice were observed to have fatty livers. Two of these animals were found to have liver tumors.

6. Fenton, P. F., and Mershon, J. S., *Fed. Proc.*, 1951, v10, 382.

Received May 28, 1951. P.S.E.B.M., 1951, v77.

Effect of Nitro-Compounds and Aldehyde Semicarbazones on Virus of Primary Atypical Pneumonia.* (18801)

MONROE D. EATON, MAUDE E. PERRY, AND ILA M. GOCKE.

From the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

Since the atypical pneumonia virus is intermediate in size between the agents of the psittacosis-LGV group and the so-called "true" viruses like influenza it may occupy in relation to chemical inhibition a strategic position one step down the scale from the "bacteria-like" viruses and rickettsiae for which many inhibitory substances have already been found. For this reason further exploration of the relation of chemical structure of certain substances to antiviral activity has been undertaken with this agent.

In a previous publication(1) it was reported that chloramphenicol had little effect on the pulmonary lesions produced in cotton rats by intranasal inoculation of the atypical pneumonia virus, but more recent studies to be reported here indicate activity in chick embryos. Because of the similarity of action of other p-nitro-benzene and 5-nitrofuraldehyde derivatives particularly those containing a semicarbazone group to that of chloramphenicol against respiratory infection in mice with viruses of the psittacosis-LGV group(2) it was considered of interest to study the effect of these substances against the atypical pneumonia virus. Experiments were also done with para-amino-benzaldehyde thiosemicarbazone and its acetyl derivative which have recently been reported to show slight inhibitory activity against vaccinia virus in mice and chick embryos(3).

Materials and methods. The methods of performing the experiments in chick embryos and cotton rats were essentially the same as those described(1). Chick embryos inoculated by the amniotic route with virus received the test substances in the yolk sac. Drugs used for the chick enbryos were sterilized by heat or filtration but for intraperitoneal injection of cotton rats the semicarbazones and thio-semicarbazones were

* This work was supported in part by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service, and the Eugene Higgins Trust.

1. Eaton, M. D., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 24.

2. Eaton, M. D., Huang, C., and Levenson, C. G., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 501.

3. Hamre, D., Bernstein, J., and Donovick, R., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 275.

TABLE I. Effect of Chloramphenicol and 5-Nitro-Furaldehyde Semicarbazone Derivatives on Growth of Atypical Pneumonia Virus in Chick Embryos.

Exp. No.	Substance	Dosage mg/egg and time interval	Atypical pneumonia strain	Treated eggs — No. in pool	Treated eggs — Result of passage to C.R.*	Control eggs — No. in pool	Control eggs — Result of passage to C.R.*
1	Chloram.	5 mg 1 hr after	De	5	1/6†	8	4/6
2			"	4	2/8†	6	4/6
3			"	5	0/6	5	5/6
4			Mac	8	0/14	9	7/13
5	NF II‡	.5 mg 2×§	"	13	0/10	6	5/12
6		.5 "	De	16	3/10	12	6/9
7	NF V‡	1 "	Mac	8	0/13	7	6/12
8		1 "	"	6	9/16	10	8/15

* No. of animals with pulmonary lesions over total tested.

† Very small pulmonary lesions.

‡ NF II is 5-nitro-2-furaldehyde 2-β hydroxyethyl semicarbazone. NF V is 5-nitro-2-furaldehyde 4-(Diethylaminopropyl) semicarbazone.

§ Two doses at 2 and 48 hr after virus.

ground and suspended in saline without heating. Dosages of drug up to one third or one half the LD_{50} were used in these experiments. Amounts given intraperitoneally to the cotton rats were recorded as milligrams per 100 of body weight. The system of recording pulmonary lesions and calculating lesion scores was the same as that used before. Lesions involving ½ to ¼ of the total volume of lung tissue were recorded as 2, those involving ¼ to ⅛ of the lung as 1, those involving less than ⅛ of the lung as ½. Lesions less than 1 mm in diameter were recorded as ± and may be considered to have doubtful significance.

Results in chick embryos with chloramphenicol and derivatives of 5-nitro-2-furaldehyde semicarbazone. In embryos treated with a single dose of 5 mg of chloramphenicol 1 hour after infection multiplication of virus was apparently inhibited since only 3 of the 34 cotton rats inoculated intranasally from treated embryos developed very small pulmonary lesions while 20 of the 31 animals receiving control material had small or large patches of pneumonia. These results are presented in Table I (experiments 1-4). Since the virus grown in chick embryos infects cotton rats only at dilutions of 10^{-1} or 10^{-2} the reduction of the amount of virus in the treated embryos was 10-fold or more. With the 2 nitrofuraldehyde compounds given in 2 doses 2 and 48 hours after the virus the effects were somewhat irregular. The method could not be expected to detect individual variation between eggs since material from the chick embryos in each experiment is pooled. In Experiment 6 where some virus was detected in the treated group, 16 eggs had been pooled (the largest group in the series) thus giving a greater chance of including one positive embryo.

Results in cotton rats with nitro- or amino cyclic aldehyde derivatives. Two experiments were done in cotton rats with 5-nitro-2-furaldehyde-4-(diethylaminopropyl) semicarbazone (NF V) and one with the 2-β-hydroxyethyl semicarbazone (NF II). As will be seen in Table II both compounds at a dosage of 8 to 10 mg/100 significantly reduced the number of animals with lung lesions, but with a dose of 6.5 mg/100 of NF V (Experiment 10) the results were less favorable. P-nitrobenzaldehyde semicarbazone (NB I Experiments 10 and 11) was included in these studies for comparison with the nitrofuraldehyde semicarbazones. The results appeared to be similar to those with the nitro-furaldehyde semicarbazone. Experiments with p-nitrobenzaldehyde thiosemicarbazone and the corresponding p-amino benzaldehyde derivative at the maximum tolerated dosage gave an apparent reduction of the pulmonary lesions but the results were not as clear cut as with the semicarbazone derivatives.

The acetyl derivative of p-aminobenzaldehyde thiosemicarbazone (AB II) was found to be less toxic for cotton rats than the other substances in this series. When this com-

TABLE II. Direct Treatment of Experimental Pneumonia in Cotton Rats.

Exp. No.	Drug*	Daily dosage mg/100 g body wt	Started at, hr	No. of animals with pulmonary lesions of 2	1	½	±	0	Avg L.S.†	Infection ratio
9	NFV	10	24	0	0	1	2	9	1.7	1/12
	0			1	4	1	2	3	12.7	6/11
10	NFV	6.5	1	0	1	1	2	4	5	2/8
	NBI	6.5	1	0	0	2	0	4	3.3	2/6
	0			1	1	4	1	1	12.5	6/8
11	NFII	8	1	0	0	1	0	6	1.4	1/7
	NBI	10	1	0	0	1	3	5	2.8	1/9
	ABI	6	1	0	0	1	1	3	3	1/5
	0			2	4	0	1	2	19	6/9
12	NBII	8	1	0	1	1	3	6	4.1	2/11
	ABI	8	1	0	1	1	1	4	5	2/7
	0			0	3	4	1	3	9.5	7/11
13	ABII	20	1	0	0	0	0	8	0	0/8
	,,	10	1	0	1	1	1	5	4.4	2/8
	,,	5	1	0	1	0	0	7	2.5	1/8
	0			0	5	3	2	5	8.8	8/15

* NBI = p-nitro-benzaldehyde semicarbazone; NBII = p-nitro-benzaldehyde thiosemicarbazone; ABI = p-aminobenzaldehyde thiosemicarbazone; ABII = p-acetylamino benzaldehyde thiosemicarbazone.

† Lesion scores represent % of pulmonary consolidation—see section on Methods and Materials.

pound was given in daily doses of 20 mg per 100 g body weight, complete suppression of the pulmonary lesions was observed in one Experiment (No. 13) but when the dosage was lowered to that used with the p-nitro- or p-amino-benzaldehyde thiosemicarbazone the results were similar to those obtained with the latter two compounds.

Discussion. Despite the failure of chloramphenicol to affect materially the course of the infection with atypical pneumonia virus in cotton rats(1) this compound was found in the present study to inhibit the growth of the virus in chick embryos. Extension of these observations to other nitro compounds revealed that two derivatives of 5-nitro-2-furaldehyde semicarbazone in contrast to chloramphenicol produced only irregular inhibition of the growth of the virus in chick embryos but did have a rather marked effect in reducing the development of pulmonary lesions in cotton rats when used at the maximum tolerated dosage. Two compounds of related structure, which have not been previously tested, p-nitrobenzaldehyde semicarbazone and thiosemicarbazone also showed some inhibitory effect in cotton rats.

The observation that p-acetylaminobenzaldehyde thiosemicarbazone when used in large doses completely suppressed the pulmonary lesions in cotton rats indicates that the nitro group is not essential to this effect. All of the compounds active in cotton rats contained the semicarbazone or thiosemicarbazone structure while chloramphenicol which had little or no activity in these animals has a different side chain. Results in some of the earlier studies with the psittacosis-LGV group of viruses were analogous to the recent findings with atypical pneumonia virus. Against the psittacosis-LGV group of viruses chloramphenicol showed greater activity in chick embryos than against experimental pneumonia in mice and this was true of other chemotherapeutic agents such as penicillin, nitro-acridine and p-nitro-benzamide(2). Nitrofuraldehyde semicarbazone showed greater activity against pulmonary infections with lymphogranuloma venereum and meningopneumonitis viruses in mice than did chloramphenicol while in chick embryos the reverse was true. These observations suggest that the semicarbazone or thiosemicarbazone group may confer some special activity against pulmonary infections and the possibility of a general physiological effect in the animal as a factor in the mode of action of these compounds should be considered.

Studies to measure the degree of inhibition by various substances of viral growth in lungs in comparison with the amount of pulmonary consolidation are being carried on.

None of the semicarbazones or thiosemicarbazones used in these studies appears to have as favorable a chemotherapeutic index as aureomycin or chloromycetin so that there is nothing to indicate their clinical usefulness in the treatment of atypical pneumonia. The observed relation of chemical structure to activity against certain viruses of large particle size including recent reports of slight activity against vaccinia in chick embryos and mice (3,4) seems of interest from the fundamental point of view and as a suggestion for further studies with other viruses in the same range of particle size.

Summary. 1. Chloramphenicol inhibited the growth of primary atypical pneumonia virus in chick embryos when injected into the yolk sac as a single dose of 5 mg one hour after amniotic inoculation of virus. Less definite inhibition of the growth of this virus in chick embryos was observed with two derivatives of 5-nitro-2-furaldehyde semicarbazone. 2. Cotton rats infected intranasally with the virus of atypical pneumonia were treated by intraperitoneal injection of several different cyclic aldehyde semicarbazone and thiosemicarbazone derivatives. Most marked suppression of the pulmonary lesions was obtained with substituted 5-nitro-2-furaldehyde semicarbazones, p-nitrobenzaldehyde semicarbazone, and p-acetylaminobenzaldehyde thiosemicarbazone.

The authors wish to acknowledge with thanks the generous supply of compounds furnished by Dr. A. F. Langlykke of the Research and Development Laboratories of E. R. Squibb and Sons, and by Dr. Mary F. Paul of the Eaton Laboratories, Inc.

Received May 31, 1951. P.S.E.B.M., 1951, v77.

4. Thompson, R. L., Price, M. D., and Minton, S. A., Jr., *Proc. Fifty-first Gen. Meeting Soc. Am. Bact.*, 1951, p. 102.

An Epizootic of Unknown Etiology in Guinea Pigs.* (18802)

E. C. PIRTLE AND A. P. MCKEE.

From Department of Bacteriology, State University of Iowa, College of Medicine.

Several workers in diagnostic and research laboratories have observed extraneous diseases among their experimental guinea pigs. Römer (1) discovered a sporadic disease in guinea pigs which he termed infectious paralysis. The virus of this disease could be passed indefinitely by brain to brain transfer. Jackson(2) described intracellular parasites in the submaxillary glands of guinea pigs, but these were shown by Cole and Kuttner(3) to be inclusion bodies. Later Kuttner(4) found these inclusions were due to a specific salivary gland virus, and succeeded in passing the virus for 3 passages to susceptible young guinea pigs. Rosenbusch and Lucas(5) encountered a salivary gland virus of pronounced virulence while engaged in a genetics experiment; the virus was peculiar in that it was pathogenic for guinea pigs by various routes of inoculation. While conducting a nutritional experiment, Pappenheimer and Slanetz(6) were confronted with a spontaneous visceral disease in their guinea pigs. The findings were suggestive of a viral etiology, although attempted

* This investigation was aided by the Central Scientific Fund, College of Medicine, State University of Iowa.

1. Römer, *Zbl. Bakt.*, 1911, v50, 30.

2. Jackson, L., *J. Infect. Dis.*, 1920, v26, 347.

3. Cole, R., and Kuttner, A. G., *J. Exp. Med.*, 1926, v44, 855.

4. Kuttner, A. G., *J. Exp. Med.*, 1927, v46, 935.

5. Rosenbusch, C. T., and Lucas, A. M., *Am. J. Path.*, 1939, v15, 303.

6. Pappenheimer, A. M., and Slanetz, C. A., *J. Exp. Med.*, 1942, v76, 299.