birds given B-12 grew more rapidly. Since better growth was obtained at lower blood levels of amino acids, it appears likely that one function of vit. B-12 is to enhance anabolic processes, which remove amino acids from the blood to form fixed tissues. More efficient utilization of blood components leading to lower blood levels could well result in reduction of renal wastage. This in turn should be reflected in greater weight gain per unit of feed; which was found to be the case in both experiments.

The high efficiency of feed utilization observed in these experiments is of interest. Mishler, Carrick and Hauge(11) reported that when a similar ration containing presumably adequate B-12 (as fish solubles) was further supplemented with 0.3% DL-methionine, the methionine gave improved growth and feed utilization. In the present studies the diets were supplemented with 0.3% of DL-methionine, and feed utilization efficiency was very high even in the absence of B-12; although still further enhanced by its presence. A practical type, high energy starter containing animal products (2.5% fish meal, 7.5% meat and bone scrap, 2.5% dried whey) has given at best about 0.45 g gain per g feed. This ration presumably contained ample B-12. Methionine supplementation may have been a requisite condition for the high feed utilization efficiency observed in the present experiments.

The blood methionine levels were unexpectedly low compared to those of the other amino acids, since examination of the amino acid composition of both chicken muscle and of the diets used showed much higher relative contents of methionine than that found in blood. Similar low methionine values (0.106 to 0.317 mg%) were reported relative to other amino acid blood levels in rats.(12) These several findings may reflect some unique relationship of methionine in metabolism, particularly as to the role of vit. B-12 in amino acid utilization.

Summary. Vit. B-12 has been shown to reduce blood levels of non-protein nitrogen and of 7 individual amino acids from the levels found in vit. B-12 deficient chicks.

Chicks given vit. B-12 grew more rapidly and utilized feed more efficiently than B-12 deficient controls, although the latter had higher blood levels of amino acids.

Vit. B-12 appears to function in metabolism by enhancing utilization of circulating amino acids for building fixed tissues.

11. Mishler, D. H., Carrick, C. W., and Hauge, S. M., *Poultry Sci.*, 1948, v27, 263.

12. Wiss, O., *Helv. Chim. Acta*, 1948, v31, 2148.

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Action of Aureomycin and Chloromycetin on the Virus of Primary Atypical Pneumonia.* (17563)

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Recently published reports of clinical studies on the treatment of primary atypical pneumonia with aureomycin(1-4) have indicated effectiveness of this antibiotic in shortening the course of the disease. Additional evidence for the effectiveness of aureomycin against the virus of atypical pneumonia will be found in the experimental studies with cotton rats and chick embryos to be reported in this paper.

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1. Schoenbach, E. B., and Bryer, M. S., *J. Am. Med. Assn.*, 1949, v139, 275.

2. Kneeland, Y., Jr., Rose, H. M., and Gibson, C. D., *Am. J. Med. Sc.*, 1949, v6, 41.

3. Finland, M., Collins, H. S., and Wells, E. B., *New England J. Med.*, 1949, v240, 241.

4. Meiklejohn, J., and Shragg, R. I., *J. Am. Med. Assn.*, 1949, v140, 391.

The observations to be recorded also help to elucidate the etiology of primary atypical pneumonia. In 1942 a virus transmissible to chick embryos, cotton rats, and hamsters was isolated from the sputum and lungs of patients with this disease.(5) Filtration experiments indicated a maximum particle size of 180 to 250 mμ for this agent(6) and it was shown to be distinct from viruses of the psittacosis group(7) and from Q fever.(8)† Evidence for the causative relationship of this virus to primary atypical pneumonia was based on the demonstration of an increase in neutralizing antibodies associated with illness in approximately 60% of the group of cases tested(8,9) which now numbers well over a hundred. The specificity of this virus neutralization was supported by the following evidence. Acute respiratory diseases such as influenza, psittacosis, and bacterial pneumonia which could be identified by clinical or laboratory methods did not stimulate production of neutralizing antibodies for the virus.(7-9) Serum from atypical pneumonia patients did not show a significant development of neutralizing antibodies for other agents including several respiratory viruses isolated from cotton rats and hamsters.(9)

Materials and methods. Virus. Two strains of the virus of atypical pneumonia, Mac and De were used in the 26th to 30th amniotic passages. The methods of passage and of preparation and storage of the virus suspensions were the same as those detailed in previous papers. Penicillin in a concentration of 100 units per ml was added routinely for the purpose of reducing contamination. The infectious titer of these suspensions averages approximately 10^{-2} in cotton rats and 10^{-3} to 10^{-4} in chick embryos.(6)

Antibiotics. Aureomycin was dissolved in saline containing 0.05 M phosphate buffer. Solutions were made up fresh daily and injected as soon as possible except that in some instances the unused portion of the solution remaining in the vial was stored at −70°C overnight and used up the next day. Chloromycetin was dissolved or suspended in plain physiological saline, complete solution being obtained only in concentrations of 5 mg per ml or less.

Experiments in cotton rats. Cotton rats of the species *Sigmodon hispidus hispidus* were obtained from a single source. The animals used in these experiments were 6 to 8 weeks of age and weighed between 60 and 80 g. Their diet consisted of Purina dog chow and water. In each experiment animals of a single shipment as uniform as possible in age and weight were divided between treated and control groups. The cotton rats were inoculated intranasally under ether anesthesia with lightly centrifuged 20% suspensions of chick embryo tissue. The antibiotics were injected once daily by the intraperitoneal route in a volume of 0.5 to 1.0 ml of solution or suspension starting 24 hours after the virus or later. The experiments were terminated 10 days after the inoculation of virus at which time all animals were sacrificed, autopsied, and the extent of the pulmonary lesions recorded. The usual system of recording lesions by gross pathology and of calculating lesion scores was used with certain modifications. 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 millimeter in diameter were recorded as ± and may be considered to have doubtful significance. In calculating the lesion scores, however, such ± lesions were arbitrarily assigned a score of ¼. The lesion scores in per cent were obtained as usual by adding together the scores for the animals in a group and dividing by 5 times the total number in the group. In representing the infection ratio of a group of animals (number having pulmonary lesions over the total inoculated) the

5. Eaton, M. D., Meiklejohn, G., and van Herick, W., *J. Exp. Med.*, 1944, v79, 649.

6. Eaton, M. D., Meiklejohn, G., van Herick, W., and Corey, M., *J. Exp. Med.*, 1945, v82, 317.

7. Eaton, M. D., PROC. SOC. EXP. BIOL. AND MED., 1945, v60, 231.

8. Eaton, M. D., and van Herick, W., *Am. J. Hygiene*, 1947, v45, 82.

† See footnote page 93 and discussion on pages 92 and 93 of reference 8.

9. Eaton, M. D., van Herick, W., and Meiklejohn, G., *J. Exp. Med.*, 1945, v82, 329.

TABLE I.
Effect of Dosage of Aureomycin in Cotton Rats, Strain De.

Exp. No.	Aureomycin dosage mg	Aureomycin dosage Started at, hr	No. of animals with pulmonary lesions of 2	1	½	±	0	Avg L.S.†	Infection ratio
1	1	24	0	1	0	1	12	1.8	1/14
	S*	"	1	5	3	2	5	11.3	9/16
2	1	"	0	0	0	0	8	0	0/8
	.5	"	1	2	1	0	3	12.8	4/7
	S	"	2	2	1	0	2	18.5	5/7
3	.5	"	0	0	1	2	4	2.9	1/7
	S	"	0	3	1	1	1	10.8	4/6
4	.5	"	0	0	2	1	4	3.6	3/7
	.2	"	0	3	2	2	0	12.8	7/7
	0	—	0	3	4	0	0	14.3	7/7
5	.2	24	0	0	2	2	3	4.2	2/7
	S	"	0	1	1	1	4	5.0	2/7

* S indicates that buffered saline was inoculated by the intraperitoneal route in place of the antibiotic. 0 indicates no inoculation.
† L.S. denotes pulmonary lesion score. See section on *Materials and Methods.*

± lesions were not counted as indicative of infection.

Experiments in chick embryos. Chick embryos inoculated by the amniotic route with the atypical pneumonia virus received aureomycin into the yolk sac. Embryos dying on the 1st to 6th day after inoculation constituted about 20 to 30% of the total groups of treated or control eggs and these were discarded. Surviving embryos were harvested 7 days after inoculation. The pooled lungs, trachea, and amniotic membranes from all eggs of the treated and control groups were made into 20% suspension. This material from the two groups of eggs was then inoculated intranasally into cotton rats to test for the presence of virus.

Results with Aureomycin in cotton rats. Determination of minimal effective dose. A series of experiments was done with aureomycin in daily doses of 0.2 to 1.0 mg per cotton rat starting treatment 24 hours after inoculation of the virus. The results are presented in Table I. Twenty-two animals were treated with aureomycin at a dosage of 1.0 mg. (Exp. 1 and 2). Only one of these developed a significant pulmonary lesion while 14 of the 21 control animals in these two experiments had lesions. The average lesion scores in the treated animals were reduced by approximately 90% as compared with the controls.

When the dosage was reduced to 0.5 mg per cotton rat daily (Exp. 2, 3 and 4) significant effects were also observed in two experiments, but a higher proportion of the animals developed pulmonary lesions and in Experiment 2 no significant difference between the control and treated group was obtained. In the 3 experiments together 8 of the 21 animals receiving a daily dose of 0.5 mg of the antibiotic developed pulmonary lesions while 16 of the 20 controls were positive. Two experiments (4 and 5) were done with aureomycin at a dosage of 0.2 mg. No difference in the incidence of infection between the treated and control animals was observed in either experiment, although the lesion score in the treated animals was slightly lower than that of the controls. The results indicate that minimal effective dose of aureomycin is approximately 0.5 mg. The average body weight of the cotton rats used in these experiments varied from 54 g in Experiment 2 to 69 g in Experiment 4 thus giving a range of 7.3 to 9.3 mg/kg of body weight at this critical dosage level.

Effect of treatment at increasing time after inoculation. In a series of 5 experiments aureomycin at a dosage of 2.0 mg per cotton

TABLE II.
Effect of Time of Starting Treatment with Aureomycin in Cotton Rats.

Exp. No.	Strain	Aureomycin dosage mg	Started at, hr	No. of animals with pulmonary lesions of 2	1	½	±	0	Avg L.S.	Infection ratio
6	Mac	2	24	0	0	0	2	7	1.1	0/9
	S		”	1	2	3	1	3	9.5	6/10
7	Dc	2	48	0	0	1	1	8	2.5	1/10
	S		”	0	3	4	1	2	10.5	7/10
8	”	2	72	0	0	0	0	12	0	0/12
	S		”	1	3	2	1	6	10.4	6/12
9	Mac	2	96*	0	0	0	0	5	0	0/5
	S		”*	0	1	2	0	2	8.0	3/5
10	De	2	120*	0	0	1	4	10	2.0	1/15
	5		144*	0	0	2	2	6	3.0	2/10
	0		—*	0	3	3	1	7	6.8	6/14

* Animals sacrificed 11 days after inoculation instead of at 10 days.

TABLE III.
Results of Treating Cotton Rats with Chloromycetin.

Exp. No.	Chloromycetin dosage mg	Started at, hr	No. of animals with pulmonary lesions of 1	½	±	0	Avg L.S.	Infection ratio
3a	5	24	0	3	1	3	5.0	3/7
	S	”	3	1	1	1	10.8	4/6
4a	5	48	2	5	0	1	10.8	7/8
	20	”	3	1	0	2	11.6	4/6
	0	—	3	4	0	0	14.3	7/7
5a	20*	24	1	0	3	3	4.4	1/7
	S	”	1	1	1	4	5.0	2/7

* Given as divided doses of 10 mg each on first 3 days.

rat daily was given at various times up to 120 hours after inoculation. The results are presented in Table II. In Experiments 6 through 9 it is evident that an increase of the interval up to 96 hours did not significantly reduce the effectiveness of the antibiotic in preventing or eliminating the pulmonary lesions. In Experiment 10 the aureomycin was given at a dose of 2.0 mg daily starting at 5 days and of 5.0 mg daily starting at 6 days after infection. In this experiment the antibiotic appeared to be somewhat less effective than when started earlier. Small lesions were found in the lungs of some of the treated animals. In two instances, however, these lesions appeared to be much greyer than usual indicating that regression had begun earlier as a result of the treatment.

Results with Chloromycetin in cotton rats. Experiments with chloromycetin were done with daily doses of 5 and 20 mg starting treatment 24 to 48 hours after intranasal inoculation of the virus. The results are presented in Table III. In Experiment 3a the incidence of infection in the treated group receiving 5.0 mg of chloromycetin per day was similar to that of the control group but the lesions in the treated animals were on the average smaller than in the controls. In Experiment 4a the lesion scores and incidence of infection in the 2 treated groups were slightly lower than the controls. In the last experiment of Table III the dose of virus was so small that only 2 of the 7 controls developed pulmonary lesions, but one of the treated animals had rather extensive consolidation and in the lungs of 3 other animals there were minute spots and diffuse hyperemia graded as ± lesions.

TABLE IV.
Inhibitory Effect of Aureomycin on Growth of Atypical Pneumonia Virus in Chick Embryos.

Exp. No.	Atypical pneumonia strain	Dosage, mg/egg and time interval	Treated eggs		Control eggs	
			No. in pool	Result of passage to C.R.*	No. in pool	Result of passage to C.R.*
11	De	0.5 mg mixed with virus†	14	0/4	17	4/5
12	"	1 mg 2 hr after virus	12	0/6	15	4/7
13	"	1 mg 2 hr after virus	11	2/5	10	2/5
14	Mac	0.8 mg 2 hr after virus	4	2/8	7	5/8
15	"	1 mg 2 hr after virus	13	4/9	11	6/6
16	De	1 mg 2x (2 and 48 hr)	7	1/11	8	8/11
17	"	1 mg 2x (2 and 48 hr)	13	0/9	10	8/9
18	Mac	0.6 mg 2x (2 and 48 hr)	18	0/7	8	4/6

* Numerator represents number of cotton rats with lung lesions, denominator number tested.
† In this one experiment aureomycin at a concentration of 10 mg/ml and an equal part of virus suspension were mixed and 0.1 ml of the mixture inoculated into amnion.

Examination of the peritoneal cavity at autopsy of cotton rats receiving chloromycetin indicated good absorption of the antibiotic since only a few small particles remained.

Results with Aureomycin in Chick Embryos. Experiments in chick embryos were done to show that aureomycin actually inhibited multiplication of this virus because the objection could be raised that the results in cotton rats might be due to inhibition of the pathological reaction in the lungs. Such experiments in chick embryos also eliminated the possibility that the observed therapeutic effect was on infection with a latent virus carried by the animals themselves. In the first experiment aureomycin was mixed at a concentration of 10 mg per ml with 20% virus suspension and 0.1 ml of the resulting mixture inoculated without further incubation into the amnion of chick embryos. Cotton rats inoculated by the intranasal route with this material developed pulmonary lesions indicating the presence of active virus in the inoculum. Tests for the presence of virus in the treated chick embryos by subinoculation to cotton rats as described in the section on materials and methods were, however, negative. Control embryos were positive for virus. These results are summarized in the first line of Table IV (Experiment 11). The results of 4 subsequent experiments in which a single dose of 0.8 to 1.0 mg of aureomycin was inoculated into the yolk 2 hours after the virus were somewhat irregular (Experiments 12, 13, 14 and 15).

More significant effects were obtained when 2 doses of aureomycin were given 2 and 48 hours after the virus. In the total of 27 cotton rats inoculated from infected chick embryos in Experiments 16, 17, and 18, only one animal developed lung consolidation while 20 of the 26 animals receiving material from the control chick embryos developed pulmonary lesions. Since the material from the entire group of 7 to 18 treated chick embryos in each experiment was pooled for the tests in cotton rats this result indicates that aureomycin produced a considerable reduction of the amount of virus in every chick embryo.

Discussion. From the results presented it appears that the daily dose of aureomycin necessary for a definite therapeutic effect on the virus of atypical pneumonia in cotton rats varies from 20 to 100 mg per kilo of body weight depending upon the time after infection at which treatment is started. This is in good agreement with the dosage reported by other workers(1-4) to be effective against the disease in human beings. The oral dosage in man was between 2 to 6 g per day or 30 to 85 mg/kg. The exact nature of the agent causing primary atypical pneumonia has not been established but filtration data(6) indicate that it may be a representative of a class of agents sensitive to aureomycin but somewhat smaller than viruses of the psittacosis-lymphogranuloma group which are also inhibited by this drug.(10). In some of the experiments in human volunteers the agent is reported to have passed a Seitz filter (11,12) indicating a still smaller particle size. Re-

10. Wong, S. C., and Cox, H. R., *Ann. N. Y. Acad. Sc.*, 1948, v51, 290.

peated microscopic examination of the infected tissues of human beings and experimental animals by a number of investigators has failed to reveal the presence of rickettsiae or elementary bodies. Although the experiments with chloromycetin in cotton rats were of a preliminary nature they leave little doubt that at a dosage up to 400 mg/kg the effects of this antibiotic against the agent of primary atypical pneumonia are irregular. The results parallel to some extent other observations with these two antibiotics against viruses of the psittacosis-lymphogranuloma group. Against intracerebral or intranasal infections of mice with these agents aureomycin apparently shows a greater activity than chloromycetin.(10,13,14)

11. Commission on Acute Respiratory Diseases, *Bull. Johns Hopkins Hosp.*, 1946, v79, 97.

12. Dingle, J. H., *Bull. N. Y. Acad. Med.*, 1945, v21, 235.

13. Smadel, J. E., and Jackson, E. B., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 478.

Summary. Aureomycin injected intraperitoneally starting 24 hours after intranasal infection of cotton rats with the virus of primary atypical pneumonia almost completely inhibits the development of pulmonary consolidation when given in daily doses of 1 mg, and is partly active at a dosage of 0.5 mg per day. Therapeutic activity with doses of 2.0 to 5.0 mg daily was also demonstrable when the beginning of treatment was delayed for 5 to 6 days.

Chloromycetin in doses of 5 to 20 mg per day had slight and irregular effects on the virus in cotton rats under the same experimental conditions.

Aureomycin inhibits the growth of the atypical pneumonia virus in chick embryos when injected into the yolk sac in two doses of 1 mg each 2 and 48 hours after amniotic inoculation of the virus.

14. Eaton, M. D., unpublished observations.

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The Huggins Cancer Test on 700 Normal Persons. (17564)

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Huggins *et al.*(1) have demonstrated that blood serum from persons with cancer, when heated at 100°C for 30 minutes, coagulates less readily than does normal serum. This defect in serum from persons with cancer can be accurately detected by the use of an inhibitor to coagulation, sodium iodoacetate, at a pH of 7.4. To permit mathematical expression of this phenomenon, Huggins proposed the "iodoacetate index"; the micro-moles of iodoacetate required to inhibit coagulation of one milliliter of serum, divided by the total protein of the serum in grams per cent. In a group of 283 individuals; 100 normals, 88 cancer patients, and 95 patients with non-malignant pathology; 85 clinically active cancers had indices of less than 9.0, as did 16 patients with non-malignant pathology. All normals fell within the range of 9.2 to 12.6. Since it is equally important to know how often one may expect an index below 9.0 in healthy persons, we have determined the iodoacetate index of 700 apparently healthy men and women of various ages at a large Army Training Center.

In our survey, the method, reagents, and equipment to determine the iodoacetate index were exactly the same as those used by Huggins and his coworkers.(2) The total serum protein was determined by the falling drop method, using the Eimer and Amend falling

1. Huggins, C. B., Miller, G. M., and Jensen, E. V., *Cancer Research*, 1949, v9, 117.

2. Huggins, C. B., Miller, G. M., and Jensen, E. V., *Simplified Procedure for Serum Coagulation Test*, University of Chicago, 24 May, 1949.