Brit. J. Pharmacol. (1950), 5, 611.

THE THERAPY OF EXPERIMENTAL PSITTACOSIS AND LYMPHOGRANULOMA VENEREUM (INGUINALE)

I. THE COMPARATIVE EFFICACY OF PENICILLIN, CHLORAMPHENICOL, AUREOMYCIN, AND TERRAMYCIN

BY

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(Received September 7, 1950)

Hurst (1948) reported the moderate therapeutic activity of nitroakridin 3582 against psittacosis in mice and lymphogranuloma venereum in developing chick-embryos. Against the strain of psittacosis virus used, its action, though not outstanding, exceeded the just perceptible effects of " Sulphamezathine " and sulphadiazine and was less than that of penicillin. Hurst also summarized earlier literature relating to the treatment of the experimental diseases with sulphonamides, penicillin, and trypaflavin. After the article had been sent to the press, there became available in this country the paper of Eaton, van Allen, and Wiener (1947) describing the activity of several nitroacridines against organisms of the psittacosis-lymphogranuloma group; in these compounds substitution of Cl for NO_2 abolished activity.

More recently, Eaton, Huang, and Levenson (1949) have demonstrated similar activity on the part of several substituted nitrobenzenes and nitrofurans. The compounds examined included the antibiotic chloramphenicol (" Chloromycetin "), which although the least toxic of the substances tested did not show striking therapeutic superiority over other nitro-compounds. In general its activity did not seem " to be greater than that of penicillin or the sulfonamides "; lymphogranuloma was among the viruses most susceptible to its action. Smadel and Jackson (1948) had previously found that chloramphenicol possesses " about the same degree of activity as that displayed by penicillin and sulfadiazine."* It was ineffective *in vitro*, but in mice it exerted a therapeutic effect against psittacosis virus given intraperitoneally (but not intracerebrally), even though a period of delay ensued between infection and treatment. In treated animals the titre of virus in the blood was lower than in controls. In developing eggs the antibiotic was rather more effective against lymphogranuloma than against psittacosis.

Wright *et al.* (1948) used another antibiotic, aureomycin, with encouraging results against human lymphogranuloma venereum. Wong and Cox (1948) found

* Against the strains of virus described in this paper, a marked difference exists in the relative activities of the sulphonamides and of penicillin; the latter is incomparably more potent. A careful comparison of our own and published data suggests also that our strains of virus are more virulent than some of those used elsewhere in chemotherapeutic experiments.

that, while showing little or no virucidal effect *in vitro*, its therapeutic action against viruses of the psittacosis-lymphogranuloma group in chick-embryos and mice was considerable. Thus, the former were protected against large inocula of psittacosis virus given into the yolk-sac 30 minutes after the antibiotic. Treated mice usually survived intraperitoneal inoculation of psittacosis virus or intracerebral inoculation of lymphogranuloma virus, even though treatment was delayed after infection. Wagner (1949) studied experimentally the action of aureomycin on ten strains of virus belonging to the psittacosis-lymphogranuloma group. In the infected egg all strains were extremely susceptible to its action, as indicated by longer survival of treated embryos and, if the infecting dose were small, by the lower titre of virus on subinoculation. The drug also prolonged life in mice infected intracerebrally with five of the strains pathogenic for this species. Comparing aureomycin with chloramphenicol in the treatment of psittacosis in the chick-embryo, Wells and Finland (1949) concluded that on a molecular basis the former is five times as effective as the latter, on a weight basis three times.

The observations on penicillin, chloramphenicol, aureomycin, and terramycin here described were made while attempting to establish increasingly exacting standards of competition for synthetic organic chemicals which show degrees of therapeutic activity against the viruses under consideration.

Strains of virus used

In this work we have used the following strains of virus:

(*a*) A lymphogranuloma virus (LGI) obtained originally from Dr. G. Rake and maintained by passage through the yolk-sac of developing hens' eggs. Each infected yolk-sac was shaken with glass beads and 2 c.c. saline to make a suspension which was stored briefly at + 2° C. In recording dilutions in Table I, we have considered the suspension as undiluted virus (10°).

By passing this virus intracerebrally and later intraperitoneally in mice, we have twice obtained a virus which infects mice on intraperitoneal injection; this was used for experiments on mice. The ability to infect on intraperitoneal inoculation is not generally recognized as characteristic of the virus of lymphogranuloma, and indeed the present virus often does not do so on repeated intraperitoneal passage. Its exact antigenic behaviour has not yet been checked, but against several chemotherapeutic agents its behaviour is different from that of the psittacosis viruses in use in these laboratories. It produces intracellular bodies indistinguishable from those of lymphogranuloma.

(*b*) The M.O.H. 154 strain of psittacosis virus (Ps) supplied by Professor S. P. Bedson and maintained by intraperitoneal injection in mice. Infected spleens were kept without glycerol at +2° C., or more recently as suspensions in sterile skimmed milk at —70° C.

(*c*) The KLG virus sent from another laboratory as a mouse-virulent lymphogranuloma virus. However, its immunological behaviour shows it to be more nearly related to psittacosis virus.* It was maintained in the same way as (*b*).

EXPERIMENTAL RESULTS

The therapy of lymphogranuloma in the chick-embryo

Table I presents the results of experiments performed over a period of about fifteen months. Two points deserve mention. In the first place the potency of different samples of virus varied, but by suitably adjusting the infecting dose we

* We are greatly indebted to Dr. C. F. Barwell for this information.

TABLE I

COMPARATIVE EFFICACY OF SULPHONAMIDES, PENICILLIN, CHLORAMPHENICOL, AUREOMYCIN, AND TERRAMYCIN AGAINST LYMPHOGRANULOMA IN THE CHICK-EMBRYO

Virus (10^{-2}–10^{-4} according to the activity of a particular sample) was inoculated into the yolk-sac of 6-day embryos. Two hours later, groups each of 19–22 embryos received 0.25 c.c. water or the stated amount of drug in water, dispersing agent (sulphonamides), or propylene glycol (chloramphenicol). In estimating the mean period of survival subsequent to infection, the few non-specific deaths were excluded. Embryos alive when the eggs were opened on the 20th day of incubation were deemed to have survived for one additional day; * indicates that 25 per cent or more survived, † 50 per cent or more. The effects on survival of the various treatments are expressed as percentages of the effect of 1 mg. aureomycin in the corresponding experiment

Treatment	Mean period of survival in days and percentage effect in terms of 1 mg. aureomycin = 100												Mean % effect
	(i)		(ii)		(iii)		(iv)		(v)		(vi)		
	Days	% effect	Days	% effect	Days	% effect	Days	% effect	Days	% effect	Days	% effect	
Water	3.8	—	4.8	—	4.0	—	4.0	—	7.4	—	4.6	—	—
Penicillin 5,000 units	—	—	—	—	5.8	40	5.7	27	—	—	—	—	34
2,000 units	—	—	—	—	5.4	31	6.5	39	—	—	—	—	35
500 units	6.2	23	6.5	22	4.5	11	—	—	—	—	—	—	19
Chloramphenicol 2.5 mg.	—	—	—	—	7.7	82	7.4	53	—	—	—	—	68
1.0 mg.	8.2	43	8.4	47	5.9	42	5.1	17	—	—	—	—	37
0.5 mg.	5.3	15	7.2	32	—	—	—	—	—	—	—	—	24
Aureomycin 2 mg.	—	—	—	—	9.1	113	10.8*	106	—	—	—	—	110
1 mg.	14.1†	100	12.4*	100	8.5	100	10.4	100	9.6*	100	10.5*	100	100
0.5 mg.	9.4	38	9.0	55	—	—	—	—	—	—	—	—	47
" Sulphamezathine " 20 mg.	—	—	—	—	4.0	—	—	—	—	—	—	—	—
10 mg.	—	—	—	—	4.0	—	—	—	—	—	—	—	—
Sulphadiazine 20 mg.	—	—	—	—	4.0	—	—	—	—	—	—	—	—
10 mg.	—	—	—	—	4.0	—	—	—	—	—	—	—	—
Terramycin 2 mg.	—	—	—	—	—	—	—	—	10.9*	159	—	—	159
1 mg.	—	—	—	—	—	—	—	—	12.5†	232	—	—	232
0.2 mg.	—	—	—	—	—	—	—	—	—	—	10.2*	95	95
0.1 mg.	—	—	—	—	—	—	—	—	—	—	11.2†	112	112

obtained very regular periods of survival in individual untreated embryos, except in Exp. v, when we greatly overestimated the activity of the virus. Thus, in the third and fourth tests all the embryos died on the 4th day, in the first test late on the 3rd day or on the 4th day, and in the second and sixth tests on the 4th or 5th days. Secondly, at different times of year the response of the embryos to treatment was not the same. At times when the fertility of the eggs was high, as in Tests (i) and (ii), a given dose of any antibiotic had a greater therapeutic effect than at times when fertility was low, as in Tests (iii)–(vi). We have noticed similar behaviour on many occasions, not only when using eggs in chemotherapeutic tests but also when grafting tumours. Because of this seasonal variability, we have in Table I set out the increase in the mean period of survival resulting from each treatment as a percentage of that resulting from a standard treatment. The effect of 1 mg. aureomycin suggests itself as the most suitable present standard.

Aureomycin had the greatest effect of any of the older antibiotics; usually, several or many of the embryos treated with it were living when opened on the day before they were due to hatch. A dose of 2 mg. was only slightly superior to one of 1 mg.; lower doses were greatly inferior, and appreciably higher doses toxic. Terramycin (obtained only recently through the courtesy of Messrs. Chas. Pfizer & Co., Inc.) appeared, in limited tests, to be superior to aureomycin when given in comparable doses and to preserve its activity when the dose was diminished. Chloramphenicol at the largest dose given came next in order of therapeutic activity; still larger doses (in propylene glycol) were toxic. Penicillin took fourth place, and probably no advantage accrued from giving a dose greater than 2,000 units/egg; doses larger than 5,000 units kill many eggs.

For comparative purposes, in one experiment we included treatments with "Sulphamezathine" and sulphadiazine: although on this occasion they failed completely to influence the infection, we have sometimes obtained a trivial prolongation of life in embryos treated with lower doses (5 mg. and 2.5 mg.) than those shown in Table I.

The therapy of psittacosis and lymphogranuloma in the mouse

The following experiments are representative of a much larger number made over the past two years. The work began before chloramphenicol and aureomycin became relatively freely available, and terramycin has arrived upon the scene still more recently; in consequence direct comparison of all the antibiotics has not been made in any single experiment.

Groups of randomized mice (20 g. $\pm$ 1 g.) received intraperitoneally very large doses of virus (10^3–10^5 LD50), and treatment was often delayed for several days after infection. We administered the antibiotics by a route other than that used for virus and observed the mice for a minimum of 35 and a maximum of 50 days. Observers elsewhere have often contented themselves with periods of observation much shorter than this. As we have previously shown (Hurst, 1948), however, on occasion animals may relapse and die apparently of the viral infection long after therapy has ceased; all our subsequent experience has emphasized the need for prolonged observation in order to avoid unduly favourable conclusions. This is, perhaps, especially the case with chloramphenicol. Thus in Test 1 of Table III, only 6 deaths had occurred by the 21st day among the animals treated with this drug, but between

TABLE II

THE ACTION OF PENICILLIN, CHLORAMPHENICOL, AUREOMYCIN, AND TERRAMYCIN ON PSITTACOSIS IN MICE

Virus was given intraperitoneally, the therapeutic agents by other routes. In different experiments the animals were observed for 35–50 days

Test No.	Treatment	Treatment begun	Duration of treatment	Mortalities in groups of 30 mice		
				Virus	Deaths	Mean period of survival of fatal cases in days
1	None	—	—	KLG 10^{-3}	30	8.8
	Penicillin in water, 500 units/20 g. subcut. 4 times daily*	4 hr. before virus	18 days		16	16.4
	Procaine-penicillin, 30,000 units/20 g. subcut. every third day	,,	,,		9	17.0
	Aureomycin, 2 mg./20 g. orally twice daily	,,	,,		0	—
	1 mg./20 g. as above	,,	,,		9	27.2
2	None	—	—	Ps 10^{-4}	26	6.2
	Penicillin in water, 500 units/20 g. as above	4 hr. before virus	18 days		11	32.7
	Procaine-penicillin, 30,000 units/20 g. as above	,,	,,		2	9.5
3	None	—	—	Ps 10^{-3}	28	7.0
	Aureomycin, 2 mg./20 g. as above	72 hr. after virus	12 days		0	—
	Chloramphenicol in propylene glycol, 1 mg./20 g. subcut. twice daily	,,	,,		22	16.0
4	None	—	—	KLG 10^{-2}	27	4.4
	Procaine-penicillin, 6,000 units/20 g. as above	When mice began to die	12 days		20 (6)†	4.9
	30,000 units/20 g. as above	,,	,,		17 (9)†	10.1
	Aureomycin, 2 mg./20 g. as above	,,	,,		17 (5)†	11.6
5	None	—	—	Ps 10^{-3}	25	5.8
	Aureomycin, 2 mg./20 g. as above	48 hr. after virus	12 days		0	—
	1 mg./20 g. as above	,,	,,		0	—
	Terramycin, 2 mg./20 g. orally twice daily				1	[24.0]
	1 mg./20 g. as above				2	16.0

* Given at 9 a.m., 12.45, 5, and 9.30 p.m. In addition, a solution of penicillin was proffered overnight from sterilized drinking-bottles.
† The figures in parentheses are the number of deaths before treatment started ; these animals were excluded in calculating the mean period of survival.

the 22nd and 39th days 18 further mice died. We have made similar observations on many occasions.

Table II shows the results obtained with the two strains of psittacosis virus. As Tests 1 and 2 indicate, we found that a massive dose of 30,000 units of procaine-penicillin* given once every three days had greater protective action than did the smaller and more frequent watery injections used in our previous work. The data of Test 4 (and of Test 2 in Table III) suggest that this enhanced action is partly or largely due to the higher total dosage, since doses of procaine-penicillin comparable with those used for watery injections had distinctly less curative power than did the higher doses. In Test 1, the larger dose of procaine-penicillin was much less successful than aureomycin was in doses of 2 mg., and about equal in activity to half this dosage of aureomycin. Test 3 demonstrates the remarkable therapeutic properties of aureomycin even when administration is delayed for 72 hours after infection; under the conditions of this experiment, it proved far more active than chloramphenicol in doses of 1 mg. Test 4, in which a very large dose of virus killed animals from the third day onwards, reveals an appreciable curative effect of both procaine-penicillin and aureomycin in animals which were not actually moribund at the time when treatment started. Test 5 suggests that aureomycin may be slightly more active than an equal amount of terramycin, but, as will be seen shortly, in a similar test with lymphogranuloma the reverse appeared to be the case.

Table III presents the results of tests with lymphogranuloma virus leading to much the same conclusions. The larger dose of procaine-penicillin proved inferior to one of 2 mg. aureomycin, and this in turn to one of 5 mg. aureomycin. Even in a dose of 2 mg., chloramphenicol was less effective than the larger dose of procaine-penicillin, and much less so than 2 mg. aureomycin. Terramycin now appeared as possibly more active than aureomycin; in Test 6 both drugs showed considerable activity even at greatly reduced dosage.

These conclusions, suggested by a general survey of Tables II and III, are perhaps more clearly appreciated from Table IV, in which the reduced mortality from each treatment is expressed as a percentage of that due to aureomycin in the same experiment. Although the figures from experiments conducted at different times and under different experimental conditions cannot be expected to agree closely, there is nothing to suggest a material difference according to whether psittacosis or lymphogranuloma is under treatment. It seems permissible where possible, therefore, to calculate the mean effects of the various treatments. This confirms the clinical impression, formed during the experiments, that in the mouse aureomycin and terramycin are very similar in their therapeutic effects, that massive doses of procaine-penicillin have a lesser effect, and that chloramphenicol runs a poor fourth in the treatment of these viral infections. Once more we should emphasize that chloramphenicol would occupy a very much more favourable position if the period of observation were restricted to, say, 21 days instead of continuing for 35–50 days as in the present work.

One further point emerges from the data of Tests 1 and 4 in Table III. In two experiments here detailed and confirmed by others not presented, combined treatment

* Procaine hydrochloride, 14 per cent (w/v); aluminium monostearate, 2 per cent (w/v).

TABLE III

THE ACTION OF PENICILLIN, CHLORAMPHENICOL, AUREOMYCIN, AND TERRAMYCIN ON LYMPHOGRANULOMA IN MICE

Virus was given intraperitoneally, the therapeutic agents by other routes. In different experiments the animals were observed for 35–50 days

Test No.	Treatment	Treatment begun	Duration of treatment	Mortalities in groups of 30 mice		
				Virus	Deaths	Mean period of survival of fatal cases in days
1	None	—	—	LGI 10^{-3}	25	6.7
	Procaine-penicillin, 30,000 units/20 g. subcut. every third day	4 hr. before virus	12 days		14	29.7
	Aureomycin, 2 mg./20 g. orally twice daily	,,	,,		1	[40.0]
	Chloramphenicol in propylene glycol, 2 mg./20 g. subcut. twice daily	,,	,,		24	26.0
	Procaine-penicillin as above + aureomycin as above				7	23.3
2	None	—	—	LGI 10^{-3}	26	6.5
	Procaine-penicillin, 6,000 units/20 g. as above	48 hr. after virus	12 days		18	19.0
	30,000/20 g. as above	,,	,,		8	33.0
	Aureomycin, 2 mg./20 g. as above	,,	,,		7	22.9
	5 mg./20 g. as above	,,	,,		4	31.3
3	None	—	—	LGI 10^{-3}	26	5.1
	Aureomycin, 2 mg./20 g. as above	72 hr. after virus	12 days		11	5.6
	Chloramphenicol, 2 mg./20 g. as above	,,	,,		22	7.0
4	None	—	—	LGI 10^{-3}	25	6.7
	Procaine-penicillin, 30,000 units/20 g. as above	72 hr. after virus	12 days		20	19.3
	Aureomycin, 2 mg./20 g. as above	,,	,,		16	20.9
	Chloramphenicol, 2 mg./20 g. as above	,,	,,		21	17.7
	Procaine-penicillin as above + aureomycin as above				23	18.9
5	None	—	—	LGI 10^{-3}	28	7.1
	Aureomycin, 2 mg./20 g. as above	72 hr. after virus	12 days		1	[35.0]
	1 mg./20 g. as above	,,	,,		8	29.8
	Terramycin, 2 mg./20 g. orally twice daily	,,	,,		1	[19.0]
	1 mg./20 g. as above	,,	,,		2	31.0
6	None	—	—	LGI 10^{-3}	27	4.6
	Aureomycin, 0.5 mg./20 g. as above	48 hr. after virus	12 days		0	—
	0.2 mg./20 g. as above	,,	,,		10	26.2
	0.1 mg./20 g. as above	,,	,,		2	28.5
	Terramycin, 0.5 mg./20 g. as above	,,	,,		0	—
	0.2 mg./20 g. as above	,,	,,		1	[9.0]
	0.1 mg./20 g. as above	,,	,,		6	17.5

TABLE IV

COMPARATIVE EFFICACY OF ANTIBIOTICS AGAINST PSITTACOSIS AND LYMPHOGRANULOMA IN THE MOUSE

The reduced mortalities following various treatments shown in Tables II and III are here expressed as percentages of the effect of 2 mg. aureomycin in the corresponding experiment. In Test 4 of Table II allowance has been made for deaths which occurred before treatment began

Treatment	Percentage effect (aureomycin 2 mg. = 100)									Mean effect
	Psittacosis test				Lymphogranuloma test					
	1	3	4	5	1	2	3	4	5	
Procaine-penicillin:										
30,000 units	70	—	123	—	46	95	—	56	—	78
6,000 units	—	—	69	—	—	42	—	—	—	56
Chloramphenicol, 2 mg.	—	—	—	—	4	—	27	44	—	25
1 mg.	—	21	—	—	—	—	—	—	—	21
Aureomycin, 5 mg. ..	—	—	—	—	—	116	—	—	—	116
1 mg. ..	70	—	—	100	—	—	—	—	74	81
Terramycin, 2 mg. ..	—	—	—	96	—	—	—	—	100	98
1 mg. ..	—	—	—	92	—	—	—	—	96	94

with procaine-penicillin and aureomycin did not lead to better results than treatment with aureomycin alone; usually, indeed, the mortality among animals under combined treatment was greater than among those receiving aureomycin alone, and on occasion even greater than among those receiving procaine-penicillin alone.

The relatively favourable results of therapy with procaine-penicillin obtain only when virus is administered intraperitoneally. If it be given by the intranasal route the proportion of mice surviving therapy is smaller, and when virus is introduced into the brain the results are very poor; it is known, of course, that penicillin does not readily traverse the blood-brain barrier. On the contrary, aureomycin is equally effective whatever the route of entry of virus. These facts are evident from the data in Table V.

TABLE V

INFLUENCE OF THE ROUTE OF INOCULATION OF VIRUS ON THE RESULTS OF THERAPY WITH PROCAINE-PENICILLIN AND AUREOMYCIN

LGI virus in a dilution of 10^{-3} was given by various routes and treatment begun 24 hours later. Dosing continued for 12 days and survivors were killed on the 45th day. The figures in parentheses are the mean periods of survival of fatal cases in days

Treatment	Mortalities in groups of 20 mice		
	Route of inoculation of virus		
	Intracerebral	Intranasal	Intraperitoneal
None	20 (5.2)	19 (13.5)	15 (6.3)
Procaine-penicillin, 30,000 units subcut. every third day	15 (6.5)	9 (22.2)	5 (27.4)
Aureomycin 2 mg./20 g. orally twice daily ..	0	1 [35.0]	1 [32.0]

TABLE VI

PSITTACOSIS VIRUS. DAILY TITRES IN THE SPLEENS OF MICE TREATED OR UNTREATED WITH ANTIBIOTICS

Virus was inoculated intraperitoneally. At the times stated, the pooled spleens of 6 mice were titrated and the dilution of virus giving 40 per cent end-points in the passage mice was derived using the method given in the text. The $\log_{10}$ dilutions are given below as positive quantities. N.V. = No virus detected

Infecting dilutions of virus	Treatment	Treatment begun	Days after infection					
			1	2	3	4	7	10
10^{-5}	None	—	3.4	4.3	6.0	7.1	4.8	All dead
	Aureomycin, 1 mg./20 g. orally twice daily	4 hr. before virus	N.V.	0.4	0.1	0.8	N.V.	N.V.
10^{-4}	None	—	3.1	5.0	6.0	6.9	6.5	
	Aureomycin, 2 mg./20 g. as above	4 hr. after virus	N.V.	N.V.	1.5	0.2	N.V.	
	Aureomycin, 0.5 mg./20 g. as above	,,	N.V.	1.0	2.8	1.8	2.2	
10^{-3}	None	—	4.4	6.1	7.2	6.5	5.1	
	Aureomycin, 1 mg./20 g. as above	4 hr. after virus	N.V.	3.2	2.0	2.2	0.8	
	Procaine-penicillin, 30,000 units/20 g. subcut. every third day	,,	2.9	3.2	4.0	3.5	0.5	
10^{-5}	None	—	2.4	3.7	5.3	6.8	5.2	
	Procaine-penicillin, 30,000 units as above	4 hr. before virus	N.V.	0.8	1.5	1.6	N.V.	
	Chloramphenicol, 1 mg./20 g. subcut. twice daily	,,	N.V.	3.5	3.5	6.6	6.7	
10^{-5}	None	—	2.5	4.1	5.9	5.7	5.2	
	Chloramphenicol, 2 mg./20 g. as above	4 hr. before virus	N.V.	3.5	2.8	4.7	5.2	

Curves of growth of psittacosis virus in the presence of antibiotics

Daily titration of psittacosis virus in the pooled spleens of groups of 6 treated and 6 untreated mice afforded confirmatory evidence of the relative potency of the various antibiotics (Table VI). Almost complete suppression of growth appeared to follow dosing with 1 mg. aureomycin begun 4 hours before infection. Begun 4 hours after infection, doses of 2 mg. markedly and doses of 1 and 0.5 mg. considerably diminished viral multiplication. While not quite as effective as aureomycin, massive doses of procaine-penicillin were also highly inhibitory especially when begun prior to infection. Once more, chloramphenicol occupied the least favourable position; indeed, at the doses given in this work it seemed only to retard the virus in attaining the same ultimate titre as in controls.

Method of computing end-points.—On the advice of our colleague, Dr. O. L. Davies, we do not employ the commonly used method of Reed and Muench (1938) in calculating end-points of virus titrations. The following graphic method gives

TABLE VII

PSITTACOSIS AND LYMPHOGRANULOMA: PERSISTENCE OF VIRUS IN THE SPLEENS OF TREATED AND UNTREATED MICE 35–50 DAYS AFTER INFECTION

The animals furnishing the spleens were dosed for 12 days except where marked*, when the period of treatment was 18 days. Ten per cent suspensions of individual spleens were each passed intraperitoneally into 3 mice

Antibiotic	Dose	Treatment begun	Virus	Presence of virus in spleens				
				Number tested	Number positive	Per cent positive	Mean per cent positive	Per cent positive according to time treatment began
None	—	—	Ps	9	3	33.3	50.0	—
	—	—	KLG	8	3	37.5		
	—	—	LGI	19	12	63.2		
Procaine-penicillin	30,000 units/20 g. every third day*	4 hr. before virus	Ps	47	13	27.7	24.1	28.9
	,, ,, ,, *	,,	KLG	28	7	25.0		
	,, ,, ,,	,,	LGI	46	15	32.6		
	,, ,, ,,	72 hr. after virus	LGI	31	4	12.9		
	6,000 units/20 g. every third day	When mice began to die	KLG	9	1	11.1		13.2
	30,000 units/20 g. every third day	,,	KLG	13	2	15.4		
Aureomycin ..	2 mg./20 g. twice daily	4 hr. before virus	LGI	28	27	96.4	65.3	96.4
	,, ,,	24 hr. after virus	KLG	29	17	58.6		
	,, ,,	48 hr. after virus	LGI	24	14	58.3		
	5 mg./20 g. twice daily	,,	LGI	24	19	79.2		58.2
	2 mg./20 g. twice daily	72 hr. after virus	LGI	32	12	37.5		
	,, ,,	When mice began to die	KLG	13	9	69.2		
Procaine-penicillin + aureomycin	30,000 units every third day and 2 mg./20 g. twice daily	4 hr. before virus	Ps	28	12	42.9	41.4	49.0
	,, ,, ,,	,,	LGI	23	13	56.5		
	,, ,, ,,	48 hr. after virus	Ps	26	13	50.0		
	,, ,, ,,	,,	LGI	27	2	7.4		35.0
	,, ,, ,,	72 hr. after virus	LGI	7	6	85.7		
Chloramphenicol	2 mg./20 g. twice daily	4 hr. before virus	LGI	6	5	83.3	95.7	
	,, ,,	72 hr. after virus	LGI	17	17	100.0		
Terramycin ..	2 mg./20 g. twice daily	72 hr. after virus	LGI	27	22	81.5	78.8	
	1 mg./20 g. twice daily	,,	LGI	25	19	76.0		

at least as accurate estimates, and at the same time allows ready comparison of a series of curves for individual irregularity due to experimental error.

Serial dilutions at logarithmic or, more often, at half logarithmic intervals are prepared from 10 per cent suspensions of the infected tissue. They are injected intraperitoneally into groups each of 6 randomized mice (20 g. $\pm$ 1 g.) of a single stock. As soon as mice begin to show symptoms they are marked. The days of death in fatal infections are recorded. Survivors which have shown no sign of illness are given a number exceeding by unity the last day of frequent specific deaths; occasional very late specific deaths also receive this score. Occasional survivors which have passed through a non-fatal infection receive a score half-way between the maximum and the average for fatal infections within their group. The total score for each group of 6 mice is then plotted against the logarithm of the dilution and a smooth curve fitted by eye. We are guided in determining the probable shape of doubtful curves by " ideal curves " drawn every six months or so from the results of 3–6 replicate titrations, within a single day, of the stock virus. From the curves so plotted we choose an end-point necessitating the least amount of extrapolation. In the above work the end-point chosen was four-tenths of the distance between maximum and minimum possible scores (40 per cent end-point).

The persistence of virus in clinically recovered mice

Although several chemotherapeutic substances enable a proportion of animals to withstand infection with psittacosis or lymphogranuloma, none is known which certainly eradicates virus from the host. We considered it important, therefore, to investigate from this point of view the newer remedies dealt with in this paper. At first we tested the pooled spleens of survivors on the 35th–50th days after infection; the mice had been treated for 12 or 18 days. Invariably the passage animals developed disease, though often after a period of incubation much longer than usual. As the latter observation could be explained by (*a*) the survival of " attenuated " or, alternatively, of partially masked virus, (*b*) the persistence of only small amounts of virus, or (*c*) the persistence of virus in only some animals, we examined large numbers of individual spleens by passing a 10 per cent suspension of each intraperitoneally into 3 mice. Individual spleens varied considerably in size, some appearing normal and others greatly enlarged; no correlation existed between the size of the organ and the results of passage summarized in Table VII.

Of the few surviving control mice, only about 50 per cent carried virus; it was impossible to say whether or not the negative spleens belonged to exceptionally resistant animals which had escaped infection in spite of the heavy inoculum of virus (10^3–10^5 LD50). Among the much larger numbers of treated mice surviving, differences in carrier-rate existed according to the antibiotic used for treatment. Thus both the figures for individual experiments and the crude overall percentage carrier-rate show that, although fewer animals lived after treatment with procaine-penicillin than with aureomycin, the penicillin-treated survivors were far less prone to carry virus. This selective carrier-rate can, of course, be explained if the more susceptible mice are the ones most likely to remain carriers, and if the more efficient aureomycin protects nearly all animals while penicillin allows the more susceptible to die. In four experiments, the detailed figures suggested that this was not the whole explanation; by considering additional animals dying in the penicillin-treated

as compared with the aureomycin-treated groups as carriers of virus, and then calculating a percentage carrier-rate on the assumption that all had survived, we found the percentages so adjusted to be 46 per cent for penicillin as against 66.6 per cent for aureomycin on a total of 78 survivors, actual or estimated, in each group. For the other antibiotics, the limited evidence available was insufficient to prove that terramycin gives an appreciably higher carrier-rate than aureomycin, but chloramphenicol once more emerged as the compound least effective against virus. As with the data for mortality already considered, a combination of procaine-penicillin and aureomycin did not afford results superior to those produced by the more effective single agent, in this case penicillin.

Continuing the analysis of the figures detailed in Table VII, we found no evidence of material differences in carrier-rate according to the virus under treatment. With penicillin and aureomycin, however, the time relative to injection of virus at which treatment was begun seemed to be important; with both drugs, survivors of animals treated 4 hours before infection showed a higher carrier-rate than did those not treated for 24 hours or more after infection. In the latter groups the proportion of mice surviving was lower, so that, again, removal of the more susceptible mice from groups in which treatment was delayed might explain the difference. Unfortunately, in only one instance were observations on the effect of different times of starting treatment contained within a single experiment, and calculations of the type used above are open to greater error. However, such calculations as we have been able to make suggest that removal of the more susceptible mice when treatment is delayed may well be the determining factor of this difference in carrier-rates. This tentative conclusion may serve as a guide to further experiment; an alternative possible explanation of the difference would be in terms of the greater antigenic stimulus provided by virus which had multiplied unhindered for 24–72 hours.

In an attempt to determine whether treatment with an antibiotic given after the peak of infection has passed can influence the carrier-rate, we infected 200 mice with psittacosis (10^2 LD50) virus. Three weeks later 36 survivors, divided into groups of 12, were left untreated or were given procaine-penicillin (5 × 30,000 units every third day) or aureomycin (2 mg. twice daily for 13 days). Four of the controls, five penicillin-treated and two aureomycin-treated mice died after treatment was begun. On the 45th day after infection, four of eight controls, one of seven penicillin-treated and one of ten aureomycin-treated animals showed virus in the spleen. These differences are not statistically significant, though the combined trend for penicillin and aureomycin is suggestive of some effect on the virus.

By these passages of individual spleens from treated animals, we proved that some mice clinically recovered from psittacosis or lymphogranuloma do not harbour residual virus; thus, included in a pool, their spleens would tend to diminish the activity of the rest (see hypothesis (*c*) above). Even with single virus-positive spleens, however, great variations exist in the time taken to evoke disease in fresh animals; often, therefore, the virus in them must be either " attenuated " or partially masked, or it must be present only in small amount. Because of the small size of many spleens, we have not yet attempted to titrate the virus present in individual organs. On the other hand, we have as yet obtained no indication that virus recovered from treated animals 35–50 days after infection is in any way altered from the original.

The recovery of virus on the 35th–50th day from the majority of mice treated with repeated doses of 2 mg. aureomycin, even when treatment started 4 hours before the animals were infected, conflicts at first sight with the striking suppression of viral growth revealed by daily titrations (Table VI). We must conclude that, despite the negative results of titration on certain days, in animals under treatment the virus survives in small amount or in inapparent form and multiplies to some extent after dosing has ceased. It may be imagined, perhaps, that some degree of immunity has been engendered during the period of treatment, and that this later multiplication of virus is usually restricted; the absence of any appreciable immunity may permit unrestricted growth of virus and be responsible for the late deaths (referred to as " relapses " in a previous paper) commented on earlier in this article. Two sets of observations bear upon these points.

Groups of 30 mice infected with a large dose (10^5 LD50) of psittacosis virus were treated with aureomycin (2 mg. twice daily) for 13 days. On the 14th day half the survivors were killed and their pooled spleens passed to fresh mice. Survivors of the remaining half were similarly treated on the 40th day. In two experiments, the pooled spleens of 14 and 10 mice on the 14th day killed respectively 10 and 2 of 12 passage mice in mean times of 9.7 and 12.0 days; 12 and 10 spleens on the 40th day killed 12 and 9 of 12 passage mice in mean times of 6.5 and 10.8 days. The spleens taken on the 40th day, therefore, apparently contained more virus than did those harvested soon after treatment ceased.

On two occasions, the pooled spleens of animals under treatment with 2 mg. aureomycin twice daily for 4 days failed to produce apparent illness within 21 days of passage to fresh mice. However, when the spleens of the first-passage mice were passed to others, psittacosis developed after a rather lengthy period of incubation. This observation suggests that, while under treatment with large doses of aureomycin, virus may persist in an " attenuated " form incapable of inducing overt disease at the first passage, rather than that it merely persists in very small amounts. That the failure of treated virus to produce overt disease is probably not due to the presence in the splenic suspensions of aureomycin (or a metabolite) was shown in tests in which normal animals were dosed twice daily for 3½ days with 2 or 5 mg. aureomycin, and their blood and spleens collected a few hours after the last dose. The blood or a splenic suspension was then mixed with a 10 LD50 psittacosis virus, and the mixture inoculated immediately into mice. The blood had no effect as compared with the blood of undosed mice. In one of two experiments the splenic suspension produced some lengthening of the incubation period as compared with undosed spleen, but all the mice inoculated developed typical psittacosis.

Summary and Conclusions

This paper compares the therapeutic activity of penicillin, chloramphenicol, aureomycin, and terramycin against psittacosis in the mouse and lymphogranuloma venereum in the mouse and chick-embryo.

In the chick-embryo both terramycin and aureomycin were highly active, the former more so. Chloramphenicol took third place and penicillin (in water) fourth in order of activity.

In the mouse infected intraperitoneally, aureomycin and terramycin were very highly and nearly equally active, massive doses of procaine-penicillin less active,

and chloramphenicol only indifferently active. This conclusion followed whether the criterion of activity was diminished mortality or actual daily measurement of the growth of virus in treated mice. A combination of aureomycin and procaine-penicillin gave results inferior to those obtained with aureomycin alone, or even inferior to those with penicillin alone. The experimental conditions in this work were often very severe, in that large infecting doses of virus were allowed a substantial part of the normal period of incubation for unhindered multiplication before treatment began.

Aureomycin was equally active whether virus was introduced intraperitoneally, intranasally, or intracerebrally, but procaine-penicillin gave its best results only with the first route of inoculation.

Many animals recovering as the result of 12 or 18 days' treatment, and appearing clinically normal on the 35th–50th day, carried active virus in the spleen. The carrier-rate varied according to the antibiotic used. With procaine-penicillin it averaged from about 13 to about 30 per cent, with aureomycin from about 60 to nearly 100 per cent. With terramycin it was about 80 per cent and with chloramphenicol nearly 100 per cent. With penicillin and aureomycin the lower figures were those for animals in which treatment had started some time after inoculation of virus, the larger figures for those in which treatment began before inoculation of virus. The difference in carrier-rate according to when treatment started, and some at least of the difference in this respect between aureomycin and penicillin, was possibly due to the elimination by death of the more susceptible animals from the groups with the lower carrier-rate.*

Although treatment with suitable doses of aureomycin almost completely suppressed the growth of virus in the mouse, after treatment had ceased a limited growth of virus occurred. That in most animals this subsequent multiplication was not unrestricted was presumably due to a mechanism, for example of immunity, not present in the untreated animal. In the period of apparent suppression by aureomycin, virus might be present in a form which while failing to produce overt disease in fresh mice at the first passage did so on continued passage.

In estimating the effect of therapeutic agents in psittacosis and lymphogranuloma, considerable importance is attached to the period of observation of treated animals. In the present work this period was never less than 35 days and often extended to 50 days. If the period of observation be limited to 21 days, unduly favourable opinions may be formed, especially of chloramphenicol after treatment with which most of the deaths occur between the 21st and 40th days.

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* *Note added in proof.* Since this was written Quan, Meyer, and Eddie (*J. infect. Dis.*, 1950, **86**, 132) have reported their inability to reduce the carrier-rate of psittacosis virus in parakeets with aureomycin or penicillin.