ACTIVITY OF CERTAIN ANTIBIOTICS AGAINST NEWCASTLE DISEASE VIRUS AND THE PR8 STRAIN OF THE INFLUENZA A VIRUS

by

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INTRODUCTION

The larger share of work dealing with the action of antibiotics has been concerned with the bacteria as test organisms rather than the viruses. Some of the newer antibiotics such as chloramphenicol, aureomycin and terramycin have been found to be active against the Rickettsia and larger viruses of the psittacosis-lymphogranuloma group, but most antibiotics have been found to be ineffective against the smaller viruses including the influenza and Newcastle disease viruses. Very little work on the combined action of antibiotics against the smaller viruses has been reported. This investigation has therefore been concerned with the individual or synergistic effect of penicillin, streptomycin, dihydrostreptomycin, chloramphenicol, aureomycin, neomycin and terramycin on the virus of Newcastle disease and the PR8 strain of influenza A virus.

HISTORICAL BACKGROUND

Much of the literature concerned with the action of antibiotics on viruses has been summarized by Lewis and Welch (1951) and Baron (1951). The older antibiotics, such as penicillin, tyrothricin, tyrocidin, gramicidin, and streptomycin have been found ineffective in the treatment of influenza (U.S. Nav. Med. Lab., 1943; Florman, Weiss, and Council, 1946; Cutting, Driesback, Helpern, Erwin, Jenkins, Proescher and Tripi, 1947; Hallaver and Faust, 1944). It was found by Parker and Diefendorf (1944) that penicillin failed to inhibit Newcastle disease virus in chick embryos. Large doses of penicillin suppress the growth of many of the

of the larger viruses in mice and chick embryos (Bedson and May, 1945; Heilman and Herrell, 1944; Parker and Diefendorf, 1944; Meiklejohn, Wagner and Beveridge, 1946) and exerts some action on certain rickettsial diseases in these animals (Greiff and Pinkerton, 1944 and 1948; Moragues, Pinkerton and Greiff, 1944). In man, large doses of penicillin seem to be of value in the treatment of psittacosis (Turgasen, 1944); Wollins, 1948; Parodi, 1944) and lymphogranuloma venereum (Willcox, 1946).

Streptomycin appears to have no inhibitory effects on the growth of influenza A and B viruses in the allantoic sac of the chick embryo (Lowell and Buckingham, 1946; Florman, Weiss and Council, 1946; McKee and Hale, 1947) or on the psittacosis-lymphogranuloma group (Hamre and Rake, 1947). However, Smadel, Jackson and Gauld (1947) and Morgan and Stevens and Snyder (1947) reported suppression of most rickettsial infections in eggs, mice and guinea pigs. Donivick and Rake (1947) found that dihydrostreptomycin was slightly less active against certain rickettsia than streptomycin. However, very little has been reported on the action of this drug against viruses.

Chloramphenicol failed to inhibit either influenza A virus, Newcastle disease virus or other smaller viruses in mice, chick embryos, guinea pigs or other animals (Smith, Joslyn, Gruhzit, McLean, Poivnor and Ehrlich, 1948; McLean, Schwab, Hillegas and Schlingman, 1949). However, Smadel and Jackson (1948) and Wells and Finland (1949) reported that it was active against the psittacosis-lymphogranuloma group of viruses in chick embryos and mice. In man, it was effective

in the treatment of both the psittacosis-lymphogranuloma group of viruses (Woodward, 1951; Fagin and Mandiberg, 1950) and the rickettsial diseases (Payne, Knaudt and Palacois, 1948; Smadel, Lion, Ley and Varela, 1949; Knight, McDermott and Ruiz-Sanches, 1949; Groot, 1949; Pincoffs, Guy, Lister, Woodward and Smadel, 1949; Carson, Gowen and Cochrane, 1949; Edwards, Irwin and Holley, 1949; Schaefer and Rashkoff, 1950; Giles and Symington, 1959).

Aureomycin, according to Kass, Lundgren and Finland (1950), did not increase the resistance of mice to the acute toxic action of the influenza virus, but others (Wong and Cox, 1948; Wells and Finland, 1949; Gogolak and Weiss, 1950; Wagner, Andrew and Watson, 1951) have reported its effectiveness against the Rickettsia and psittacosis-lymphogranuloma group in chick embryos, mice and guinea pigs. There is some difference of opinion about the activity of aureomycin against the influenza virus in man. Finland, Wells, Collins and Gocke (1950) used aureomycin in eighteen cases of influenza and reported prompt alleviation of symptoms. Goldman (1950), and Stax and Wright (1949) reported similar results. However, Thallman, Kempr, Worrall and Meiklejohn (1950) reported contradictory findings. It should be noted that the dosage used by the latter workers was considerably less than that used by Finland, et al. Sheehan (1950) found that a patient who had contact with poultry and had developed a conjunctivitis due to Newcastle disease virus responded favorably to aureomycin therapy. This drug was found to be effective in the treatment of both the psittacosis-lymphogranuloma group of viruses (Wright, Saunders, Logan, Prigot and Hill, 1948; Fletcher, Siegel and Zintel, 1951; Woodward, 1950; Brainard, Lennette,

Meiklejohn, Bruyn and Clark, 1949) and certain rickettsial diseases in man (Knight, 1949; Benhamou, Destaing, Gauther and Sorrel, 1949; Schoenback, 1949; Ross, 1948; Wong and Cox, 1948).

Terramycin given subcutaneously or orally did not increase the resistance of mice or rats to the acute toxic action of the influenza virus. (Kass, Lundgren and Finland, 1950; Kass, Barnes and Finland, 1950; Vinson and Walsh, 1950). Quigland and Francis (1951) also obtained similar results in mice and chick embryos. Kass, Lundgren and Finland (1950) and Vinson and Walsh (1950) on the other hand, stated that high concentrations of terramycin inhibited infection of the PR8 strain of influenza in chick embryos. This drug had a viricidal action if added one to three hours before or one hour after the virus was introduced into the allantoic cavity. In preliminary trials terramycin was effective against psittacosis (Rose, 1950) and many rickettsial diseases in mice and chick embryos (Snyder, 1950). Kass, Barnes and Finland (1950) reported good results in the treatment of five cases of influenza. In man, terramycin was effective in treatment of the psittacosis-lymphogranuloma group of viruses (Wright, Whitaker, Wilkinson and Bienfield, 1951; Hurst, Peters and Melvin, 1951) and the rickettsial diseases (Knight, 1950; Killaugh and Magill, 1951; Bauer, Parker, Hall, Benson, Joslyn, Hightower, Snyder, Benable and Woodward, 1950; Rose, 1950; Bailey, Ley, Diercks, Lewthwaite and Smadel, 1951; Giunchi, 1951).

While the above antibiotics have received the most study there are some others which are also of interest. Waksman, Katz and Lechevalier

(1950) and Felsenfeld, Volini, Sachiko, Bachman, and Young (1950) found neomycin to be generally ineffective against the larger and smaller viruses. Salle (1950) found that subtilin inactivated influenza A and Newcastle disease virus.

There are other substances besides antibiotics which are inhibitory to the influenza virus. Nitroacridin 3582 has been reported by Green, Rasmussen and Smadel (1946) to have an inhibitory effect on the growth of influenza B in the allantoic sac of chick embryos. This was confirmed by McClelland and Van Rooyen (1948). However, Rasmussen (1951) observed that it was not effective against influenza in mice. Apple pectin and gum arabic, when injected before or after the virus, was found to inhibit the multiplication of influenza in yolk sacs (Green and Wooley, 1947) Also various lactones produced marked therapeutic effect against influenza (Rubin and Giarman, 1947).

METHODS AND MATERIALS

Newcastle disease virus was obtained from Dr. William J. Pistor (University of Arizona) and the PR8 strain of influenza A virus was obtained in lyophilized form from Dr. Carl DeBoer (Ciba Pharmaceutical Products, Inc.). The Webster strain of mice and New Hampshire Red chicks were used as the experimental animals. Food for the mice containing all necessary nutritional requirements was secured in pellet form from Rockland Farms. The eggs were obtained from the poultry department (University of Arizona). The antibiotics used were obtained from the following companies: aureomycin-hydrochloride, the intravenous form, from Lederle Laboratories; dihydrostreptomycin-sulfate from Schenley Laboratories; streptomycin-sulfate from Abbott Laboratories; neomycin-sulfate from

Upjohn and Co.; terramycin-hydrochloride, the intravenous form from Charles Pfizer and Co.; penicillin, in which one sample contained 300,000 units of procaine penicillin and 100,000 units of buffered penicillin, from Parke-Davis and Co.; chloramphenicol in capsule form dissolved in acetyl dimethylamine, and in a suspension for intramuscular use from Parke-Davis and Co. The chloramphenicol in capsule form was dissolved in both saline and propylene glycol. The former was designated as #1 and the latter #2. The drug dissolved in acetyl dimethylamine was called #3 and the chloramphenicol suspension, #4.

The experiments may be divided into three major groups; the first one in which chick embryos were used, second and third in which mice and chicks were used respectively as the experimental animals. Both the Newcastle disease virus and influenza virus were grown in the allantoic fluid of chick embryos, and in addition, the influenza virus was instilled intranasally into mice. Various antibiotics were then tested against these viruses. Chicks were inoculated with Newcastle disease virus to determine conclusively the identity of this virus.

Before the Newcastle disease virus inoculum was used in the experiments, it was passed through chick embryos several times to stabilize its infectivity. Thereafter, each inoculum used for infecting chick embryos was obtained from the infected allantoic fluid of the ones in the preceding experiment. Mice were inoculated intranasally with the lyophilized form of the influenza virus and thereafter both mice and chick embryos were infected with macerated mouse lung tissues of the mice from the preceding experiment. In one section of the chick embryo experiments the virus inoculum was undiluted and in the other the virus

inoculum was diluted before it was mixed with the antibiotic. In the mice experiments, the virus inoculum was a 10^{-6} dilution of an infected mouse lung. However, the original starting lung material may not have been the same from one mouse to the next. The antibiotics were used in concentrations recommended by the particular company producing them.

In general, the methods used in the chick embryo experiments were essentially those employed by Hirst (1948). The fertile eggs were washed, incubated for 12 days, and inoculated with the test substances into the allantoic sac. Usually the inoculum consisted of a mixture of the virus and antibiotic, except in the case of chloramphenicol which is insoluble in aqueous solution and therefore was inoculated separately. Eight to twelve groups consisting of 5 eggs each were used in each experiment. Groups 1,2,3,4,5, and 6 were, in most cases, inoculated with Newcastle disease and groups 7,8,9,10,11, and 12 with influenza virus, but at times there was deviation from this schedule. Both drug-free and virus-free controls were run simultaneously with all of the experiments.

For inoculation of the chick embryos a hand drill was first of all used to make a small opening in the shell of the egg but not the membrane underneath. This was done just above the lower part of the air sac and the whole area was then painted with iodine. The virus was next mixed with an antibiotic or antibiotics and was immediately injected with a tuberculin syringe into the allantoic fluid. A total of one-tenth milliliter per egg was used for inoculum. The openings were then covered with collodion and the eggs were incubated at 37 C for 48 hours. They were next put into the refrigerator for several hours in order to stop blood circulation while harvesting.

In order to harvest the allantoic fluid, the shell around the air sac was first removed by use of a hand drill and forceps, care being taken not to rupture the chorio-allantoic membrane. The latter membrane of each egg was then pulled back and the infected allontoic fluid poured into sterile tubes, which were numbered and stored in the refrigerator. The following day the allantoic fluids were tested for the presence of virus by the use of the hemagglutination test.

The Newcastle disease virus hemagglutination test was based on the methods used by the Division of Veterinary Science, University of California, which in turn was based on the work of Burnett (1941). For comparative purposes, the same techniques were also used in the case of the influenza virus, since they differed only in minor details from that devised by Hirst (1941). Fresh chicken blood for the hemagglutination test was obtained from healthy adult chickens by radial vein puncture and was collected in sterile tubes containing five percent sodium citrate. The cells, which were used within one hour after collection, were washed three times with physiological saline, after which they were resuspended in saline in approximately one percent concentration.

In each group of five eggs, the allantoic fluids from three eggs and two eggs were combined into two groups so that a duplicate check could be run. These duplicates were averaged and recorded in the experimental tables as a single run. Wasserman racks and tubes were used for the serological tests. Seventy-five hundredths of a milliliter of physiological saline were added to the first tube, and five-tenths to all the rest. Twenty-five hundredths of a milliliter of allantoic fluid were added to the first tube; after mixing, five-tenths of a milliliter from the first tube was transferred to the second tube. The fluid was

then serially diluted through the second to the last tube from which five-tenths of a milliliter was discarded. The last tube was used as a virus free control. Five-tenths of a milliliter of the chicken erythrocyte suspension was then added to each tube. After the tubes were shaken they were incubated at room temperature for thirty to thirty-five minutes or until the cells in the control settled out. The reaction was then read by viewing the pattern of red cells settling on the bottom of the tube. If a discrete button-like disk with smooth edges was formed, the test was read as negative. If the edges around the button were ragged, or the button as a whole diffused, the reaction was read as positive. A positive reaction in an individual tube indicated presence of the virus. If the titer of the allantoic fluid from the treated embryos was distinctly less than that of the untreated, inhibition by the antibiotic was indicated.

The experimental procedures were performed regularly on a weekly schedule. Every Friday the fertile eggs were obtained from the University of Arizona Farm and incubated for 12 days, every Wednesday the eggs were inoculated, every Friday the allantoic fluid was harvested and every Saturday the red blood cells from the chickens were collected and the hemagglutination tests performed.

In the second series of experiments, one-tenth milliliter of influenza virus inoculum was instilled intranasally into mice after they had been etherized. Five to seven mice were used in each group and the individual groups received different antibiotics or combinations of antibiotics. The latter were given by intraperitoneal injection three times a

day at approximately nine A.M., four P.M., and eleven P.M . Virus free and drug free controls were run simultaneously with the experiment. When the animals died, or were sacrificed, autopsies were performed and the lungs were checked for consolidation. The amount of consolidation was equal to the degree of infectivity of the virus, and a scale of from zero to four was used to express the relationship (Reed and Muench, 1938; Lauffer and Miller, 1944). The effectiveness of the drug was determined by its ability to prolong the life of the mice and to decrease the severity of the infection.

In the third series of experiments, the chicks were inoculated by two routes, intranasally and intramuscularly, with two-tenths of a milliliter and one-tenth of a milliliter, respectively, of infected allantoic fluid. These animals were sacrificed after symptoms of the disease appeared. The lung, liver, spleen, and in some cases brain tissues were macerated, pooled and inoculated into twelve day chick embryos. After 48 hours incubation, the presence of virus was determined by the hemagglutination test.

RESULTS

Tables nos. 1 to 25 give the results from the work using chick embryos as experimental animals. Where the word "whole" is used in the column of virus concentrations, it denotes a concentration which gave a hemagglutination titer of approximately 10^{-3}. This was a standard material from which all dilutions were made. All dilutions for the antibiotics were made from a starting material of one gram except in the case of penicillin. In this case a solution containing 400,000

units was the material for starting the dilutions. All concentrations expressed were those resulting after the drug and virus were mixed.

Table 34 summarizes the results from the chick embryo experiments. A slight inhibition of the virus was recorded for a particular drug or drug combination, if the averaged results from all the experiments gave a hemagglutination titer of two to three dilutions less than the control. If the inhibition was greater than this, the drug or drug combination was recorded as giving a moderate to complete inhibition.

When used singly, penicillin, streptomycin and neomycin had no inhibitory action against Newcastle disease virus or influenza virus in chick embryos at the concentrations tested. Aureomycin and terramycin when tried individually had only slight activity against the influenza virus and none against the Newcastle disease virus at the concentrations used. When chloramphenicol was dissolved in acetyl dimethylamine and propylene glycol, it inhibited both viruses to a great extent.

When the drugs were used in combination, chloramphenicol #2 plus aureomycin and chloramphenicol #3 plus dihydrostreptomycin seemed to give the best results. They inhibited the Newcastle disease virus and influenza virus to a large degree. However, the inhibition produced by the combination of these drugs was no greater than that of chloramphenicol alone. Therefore, synergism did not appear to exist between aureomycin or dihydrostreptomycin and chloramphenicol. Terramycin seemed to inhibit the action of chloramphenicol, and synergism between aureomycin and neomycin was apparent when low concentrations of virus were tested; however, too few experiments were performed with these combinations to be conclusive. Dihydrostreptomycin plus terramycin and neomycin plus

terramycin inhibited the Newcastle disease virus to a slight degree. This might be interpreted as synergism since neither of the drugs used alone exhibited any inhibitory action against the virus. With the exception of these latter drugs, no other indications of synergism or antagonism between the various antibiotics were noted. When injected alone, the drugs at the concentrations tested in the experiments, were non-toxic for chick embryos for the growth period of the virus.

The tables nos. 26 to 33 give the results from the work using mice as experimental animals. In these experiments none of the antibiotics used singly or in combination gave any significant inhibition against the influenza virus as measured by the survival time. However, some drug or drug combinations seemed to be slightly better than others when the degree of infectivity of the treated mice was compared. This is summarized in table 35. A scale of 0 to 4 is used to express the infectivity average and denotes the degree of infection as measured by the amount of consolidation of the lungs. The only drugs or drug combinations giving a slightly lower infectivity average were dihydrostreptomycin, aureomycin plus dihydrostreptomycin, and dihydrostreptomycin plus neomycin. One might conclude from these results that dihydrostreptomycin does have slight inhibitory powers against the influenza virus in mice.

Chicks were used as an experimental animal in order to confirm the identity of Newcastle disease virus. The embryo-adapted virus required three passages through the chicks before it caused the characteristic symptoms to appear. Almost all of the seventy-two chicks, used for the third passage of the virus, developed symptoms within three days and

died shortly after. Following the second virus passage, the macerated and pooled liver, lung and spleen tissues gave negative tests for the presence of the virus. Following the third passage, each of the tissues were tested for the presence of the virus. The organism was found to be present in the brain and the spleen but not in the lung or liver tissues.

Table 1

EXPERIMENT 1*

The Effect of Aureomycin on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	-----	
2	"	1/2	Aureomycin	$4x10^{-2}$
3	"	1/4	"	$5 x10^{-2}$
4	"			
5	"			
6	"			
7	Influenza	Whole	-----	
8	"	1/2	Aureomycin	$4x10^{-2}$
9	"	1/4	"	$5x10^{-2}$
10	"			
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x					
2	x	x	x	x	x	x	x					
3	x	x	x	x	x	x	x					
4												
5												
6												
7	x	x	x	x	x	x						
8	x	x	x	x	x	x	x					
9	x	x	x	x	x	x	x					
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 2

EXPERIMENT 2 *
The Effect of Chloramphenicol #1 on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	------	
2	"	1/2	Chloramphenicol #1	$2x10^{-4}$
3	"	1/2	"	$2x10^{-3}$
4	"			
5	"			
6	"			
7	Influenza	Whole	------	
8	"	1/2	Chloramphenicol #1	$2x10^{-4}$
9	"	1/2	"	$2x10^{-3}$
10	"			
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x	x			
2	x	x	x	x	x	x	x	x				
3	x	x	x	x	x	x	x	x				
4												
5												
6												
7	x	x	x	x	x	x	::					
8	x	x	x	x	x	x	x					
9	x	x	x	x	x	x	x					
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

T able 3

EXPERIMENT 3 *
The Effect of Chloramphenicol #3 on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	10^{-9}	------	
2	"	10^{-7}	Chloramphenicol #3	1/4
3	"	10^{-8}	"	1/4
4	"	10^{-9}	"	1/4
5	"			
6	"			
7	Influenza	10^{-7}	------	
8	"	10^{-5}	Chloramphenicol #3	1/4
9	"	10^{-6}	"	1/4
10	"	10^{-7}	"	1/4
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x											
3	x	x										
4												
5												
6												
7	x	x	x	x	x	x	x	x				
8	x	x	x									
9	x											
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

EXPERIMENT 4*
The Effect of Dihydrostreptomycin on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	------	
2	"	1/2	Dihydrostreptomycin	$4x10^{-4}$
3	"	1/2	"	$4x10^{-3}$
4	"			
5	"			
6	"			
7	Influenza	Whole	------	
8	"	1/2	Dihydrostreptomycin	$4x10^{-4}$
9	"	1/2	"	$4x10^{-3}$
10	"			
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x	x	x		
2	x	x	x	x	x	x	x	x	x	x		
3	x	x	x									
4												
5												
6												
7	x	x	x									
8	x	x	x	x	x	x	x					
9												
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 5

EXPERIMENT 5*

The Effect of Dihydrostreptomycin on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	------	
2	"	1/2	Dihydrostreptomycin	$4x10^{-2}$
3	"	1/2	"	$4x10^{-3}$
4	"	1/2	"	$4x10^{-4}$
5	"			
6	"			
7	Influenza	Whole	------	
8	"	1/2	Dihydrostreptomycin	$4x10^{-2}$
9	"	1/2	"	$4x10^{-3}$
10	"	1/2	"	$4x10^{-4}$
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x	x			
2	x	x	x	x	x	x	x	x				
3	x	x	x	x	x	x	x					
4	x	x	x	x	x	x	x	x	x			
5												
6												
7												
8												
9												
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 6

EXPERIMENT 6 *

The Effect of Streptomycin on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	------	
2	"	1/2	Streptomycin	10^{-6}
3	"	1/2	"	10^{-6}
4	"	1/2	"	10^{-6}
5	"	1/2	"	10^{-6}
6	"			
7	Influenza			
8	"			
9	"			
10	"			
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x	x	x	x	x	x			
3	x	x	x	x	x	x	x					
4	x	x	x	x	x							
5												
6												
7												
8												
9												
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 7

EXPERIMENT 7 *
The Effect of Streptomycin on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	------	
2	"	1/2	Streptomycin	$4x10^{-5}$
3	"	1/2	"	$4x10^{-5}$
4	"	1/2	"	$4x10^{-5}$
5	"			
6	"			
7	Influenza			
8	"			
9	"			
10	"			
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x	x			
2	x	x	x	x	x	x	x	x	x			
3	x	x	x	x	x	x	x	x	x			
4	x	x	x	x	x	x	x	x	x			
5												
6												
7												
8												
9												
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 8

EXPERIMENT 8* -- The Effect of Aureomycin and Terramycin, used Singly and in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$1x10^{-1}$	-------	
2	"	$1x10^{-1}$	Aureomycin	$8 x10^{-3}$
3	"	$1x10^{-1}$	Terramycin	$2x10^{-2}$
4	"	$1x10^{-1}$	Combination (equal amounts of aureomycin and terramycin)	
5	"			
6	"			
7	Influenza	$1x10^{-1}$	-----	
8	"	$1x10^{-1}$	Aureomycin	$8x10^{-3}$
9	"	$1x10^{-1}$	Terramycin	$2x10^{-2}$
10	"	$1x10^{-1}$	Combination (equal amounts of aureomycin and terramycin)	
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x	x	x						
3	x	x	x	x	x	x	x	x	x	x		
4	x	x	x	x	x	x	x	x	x	x		
5												
6												
7	x	x	x	x	x	x	x	x				
8	x	x	x	x	x	x						
9	x	x	x	x	x	x	x	x				
10	x	x	x	x	x							
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 9

EXPERIMENT 9 *
The Effect of Aureomycin and Terramycin, used Singly and in Combination, on Newcastle Disease Virus and Influenza Virus

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	------	
2	"	1/2	Aureomycin	8×10^{-3}
3	"	1/2	Terramycin	2×10^{-2}
4	"	1/2	Combination (equal amounts of aureomycin and terramycin)	
5	"			
6	"			
7	Influenza			
8	"			
9	"			
10	"			
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x	x	x	x	x	x			
3	x	x	x	x	x	x	x					
4	x	x	x	x	x	x	x	x	x			
5												
6												
7												
8												
9												
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

EXPERIMENT 10* -- The Effect of Dihydrostreptomycin and Aureomycin, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	------	
2	"	1/2	Dihydrostreptomycin and Aureomycin	$3x10^{-1}$ $3x10^{-2}$
3	"	3/4	Dihydrostreptomycin and Aureomycin	$3x10^{-2}$ $3x10^{-3}$
4	"			
5	"			
6	"			
7	Influenza	Whole	------	
8	"	1/2	Dihydrostreptomycin and Aureomycin	$3x10^{-1}$ $3x10^{-2}$
9	"	3/4	Dihydrostreptomycin and Aureomycin	$3x10^{-2}$ $3x10^{-3}$
10	"			
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x	x	x	x	x				
3	x	x	x	x	x	x	x	x	x			
4												
5												
6												
7	x	x	x	x	x							
8	x	x	x	x	x							
9	x	x	x									
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

EXPERIMENT 11* -- The effect of Streptomycin and Chloramphenicol #1, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	------	
2	"	1/2	Streptomycin and	$8x10^{-2}$
		1/4	Chloramphenicol #1	$3x10^{-2}$
3	"	1/2	Streptomycin and	$1x10^{-2}$
4	"	1/4	Chloramphenicol #1	$4x10^{-2}$
5	"			
6	"			
7	Influenza	Whole	------	
8	"	1/2	Streptomycin and	$8x10^{-2}$
		1/4	Chloramphenicol #1	$3x10^{-2}$
9	"	1/2	Streptomycin and	$1x10^{-2}$
		1/4	Chloramphenicol #1	$4x10^{-2}$
10	"			
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x	x			
2	x	x	x	x	x	x	x	x				
3	x	x	x	x	x	x	x	x				
4												
5												
6												
7	x	x	x	x	x							
8	x	x	x	x	x	x						
9	x	x	x	x	x	x						
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 12

EXPERIMENT 12*-- The Effect of Terramycin and Neomycin, Singly and in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	Whole	------	
2	"	1/2	Terramycin	$2x10^{-2}$
3	"	1/2	Neomycin	$1x10^{-2}$
4	"	1/2	Combination (equal amounts of terramycin and neomycin)	
5	"			
6	"			
7	Influenza	Whole	------	
8	"	1/2	Terramycin	$2x10^{-2}$
9	"	1/2	Neomycin	$1x10^{-2}$
10	"	1/2	Combination (equal amounts of terramycin and neomycin)	
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x	x	x	x					
3	x	x	x	x	x	x	x					
4	x	x	x	x	x	x						
5												
6												
7	x	x	x	x	x	x	x					
8	x	x	x	x	x							
9	x	x	x	x	x	x						
10	x	x	x	x	x							
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 13

EXPERIMENT 13*
The Effect of Aureomycin on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-1}$	-----	
2	"	$2x10^{-2}$	Aureomycin	$8x10^{-3}$
3	"	$2x10^{-3}$	"	$8x10^{-3}$
4	"	$2x10^{-4}$	"	$8x10^{-3}$
5	"			
6	"			
7	Influenza	$2x10^{-1}$	-----	
8	"	$2x10^{-2}$	Aureomycin	$8x10^{-3}$
9	"	$2x10^{-3}$	"	$8x10^{-3}$
10	"	$2x10^{-4}$	"	$8x10^{-3}$
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x	x			
2	x	x	x	x	x	x	x	x	x			
3	x	x	x	x	x	x	x	x	x			
4	x	x	x	x	x	x	x	x	x			
5												
6												
7	x	x	x	x	x	x	x	x	x			
8	x	x	x	x	x	x	x	x				
9	x	x	x	x	x	x	x					
10	x	x	x	x	x	x	x					
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 14

EXPERIMENT 14* -- The Effect of Chloramphenicol #2 and Aureomycin, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	10^{-9}	-----	
2	"	10^{-7}	Chloramphenicol #2 and Aureomycin	1/4 1x10-3
3	"	10^{-8}	Chloramphenicol #2 and Aureomycin	1/4 1x10-3
4	"	10^{-9}	Chloramphenicol #2 and Aureomycin	1/4 1x10-3
5	"			
6	"			
7	Influenza	10^{-7}	-----	
8	"	10^{-5}	Chloramphenicol #2 and Aureomycin	1/4 1x10-3
9	"	10^{-6}	Chloramphenicol #2 and Aureomycin	1/4 1x10-3
10	"	10^{-7}	Chloramphenicol #2 and Aureomycin	1/4 1x10-3
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	-											
3	-											
4	-											
5												
6												
7	x	x	x	x	x	x	x					
8	x	x	x									
9	-											
10	x	x	x	x	x	x						
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 15

EXPERIMENT 15 * -- The Effect of Aureomycin and Neomycin, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-9}$	-----	
2	"	$2x10^{-7}$	Aureomycin and Neomycin	$4x10^{-3}$ $2x10^{-2}$
3	"	$2x10^{-8}$	Aureomycin and Neomycin	$4x10^{-3}$ $2x10^{-2}$
4	"	$2x10^{-9}$	Aureomycin and Neomycin	$4x10^{-3}$ $2x10^{-2}$
5	"			
6	"			
7	Influenza	$2x10^{-7}$	-----	
8	"	$2x10^{-5}$	Aureomycin and Neomycin	$4x10^{-3}$ $2x10^{-2}$
9	"	$2x10^{-6}$	Aureomycin and Neomycin	$4x10^{-3}$ $2x10^{-2}$
10	"	$2x10^{-7}$	Aureomycin and Neomycin	$4x10^{-3}$ $2x10^{-2}$
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x					
2	x	x	x	x	x	x	x					
3	x											
4												
5												
6												
7	x	x	x	x	x	x	x					
8	x	x	x	x	x	x	x	x				
9	x	x	x	x	x	x	x					
10	x	x	x	x								
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive nemagglutination.

Table 16

EXPERIMENT 16 * -- The Effect of Aureomycin and Penicillin, used Singly and in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-6}$	-----	
2	"	$2x10^{-6}$	Aureomycin	$4x10^{-3}$
3	"	$2x10^{-6}$	Penicillin	1/10
4	"	$2x10^{-6}$	Combination (equal amounts of aureomycin & penicillin)	
5	"	$2x10^{-7}$	-----	
6	"			
7	Influenza	$2x10^{-6}$	-----	
8	"	$2x10^{-6}$	Aureomycin	$4x10^{-3}$
9	"	$2x10^{-6}$	Penicillin	1/10
10	"	$2x10^{-6}$	Combination (equal amounts of aureomycin & penicillin)	
11	"	$2x10^{-7}$	-----	
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x					
2	x	x	x	x	x	x	x					
3	x	x	x	x	x	x	x					
4	x	x	x	x	x	x	x	x				
5	x	x	x	x	x	x						
6												
7	x	x	x	x	x	x	x					
8	x	x	x									
9												
10												
11	x	x	x	x	x	x						
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 17

EXPERIMENT 17 *-- The Effect of Aureomycin and Penicillin, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-5}$	-----	
2	"	$2x10^{-6}$	Aureomycin and Penicillin	$4x10^{-3}$ 1/10
3	"	$2x10^{-7}$	Aureomycin and Penicillin	$4x10^{-3}$ 1/10
4	"	$2x10^{-8}$	Aureomycin and Penicillin	$4x10^{-3}$ 1/10
5	"			
6	"			
7	Influenza	$2x10^{-5}$	-----	
8	"	$2x10^{-6}$	Aureomycin and Penicillin	$4x10^{-3}$ 1/10
9	"	$2x10^{-7}$	Aureomycin and Penicillin	$4x10^{-3}$ 1/10
10	"	$2x10^{-8}$	Aureomycin and Penicillin	$4x10^{-3}$ 1/10
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x	x	x	x	x				
3	x	x	x	x	x	x	x	x	x			
4	x	x	x	x	x	x	x	x				
5												
6												
7	x	x	x	x	x	x	x	x				
8	x	x	x	x	x	x	x	x				
9	x	x	x	x	x	x	x	x				
10	x	x	x	x	x	x	x	x				
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

EXPERIMENT 18 * — The Effect of Aureomycin and Terramycin, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-4}$	-----	
2	"	$2x10^{-5}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
3	"	$2x10^{-6}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
4	"	$2x10^{-7}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
5	"	$2x10^{-8}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
6	"			
7	Influenza	$2x10^{-4}$	-----	
8	"	$2x10^{-5}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
9	"	$2x10^{-6}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
10	"	$2x10^{-7}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x							
2	x	x	x	x	x							
3	x	x	x	x	x	x						
4												
5			x									
6												
7	x	x	x	x	x	x						
8	x	x	x	x	x	x	x	x	x			
9	x	x	x	x	x	x	x					
10	x	x	x	x	x	x	x	x	x			
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

EXPERIMENT 19 * -- The Effect of Aureomycin and Terramycin, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-6}$	------	
2	"	$2x10^{-6}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
3	"	$2x10^{-7}$	------	
4	"	$2x10^{-7}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
5	"			
6	"			
7	Influenza	$2x10^{-6}$	------	
8	"	$2x10^{-6}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
9	"	$2x10^{-7}$	------	
10	"	$2x10^{-7}$	Aureomycin and Terramycin	$4x10^{-3}$ $8x10^{-2}$
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x	x			
2	x	x	x	x	x	x	x					
3	x	x	x	x	x	x	x	x	x	x		
4	x	x	x	x	x	x	x	x	x			
5												
6												
7	x	x	x	x	x	x	x					
8	x	x	x	x	x	x	x	x				
9	x	x	x	x	x	x	x	x	x	x		
10	x	x	x	x	x	x	x	x	x			
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 20

EXPERIMENT 20* -- The Effect of Chloramphenicol #2 and Terramycin, used Singly and in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-8}$	-----	
2	"	$2x10^{-8}$	Chloramphenicol #2	1/4
3	"	$2x10^{-8}$	Terramycin	$8x10^{-2}$
4	"	$2x10^{-8}$	Combination (equal amounts of chloramphenicol #2 and terramycin)	
5	"			
6	"			
7	Influenza	$2x10^{-6}$	-----	
8	"	$2x10^{-6}$	Chloramphenicol #2	1/4
9	"	$2x10^{-6}$	Terramycin	$8x10^{-2}$
10	"	$2x10^{-6}$	Combination (equal amounts of chloramphenicol #2 and terramycin)	
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x	x			
2												
3	x	x	x	x	x	x						
4	x	x	x	x	x	x	x					
5												
6												
7	x	x	x	x	x	x	x					
8												
9	x	x	x	x	x	x	x					
10	x	x	x	x								
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

EXPERIMENT 21 * -- The Effect of Chloramphenicol #2 and Terramycin, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-9}$	-----	
2	"	$2x10^{-7}$	Chloramphenicol #2 and Terramycin	1/4 $8x10^{-2}$
3	"	$2x10^{-8}$	Chloramphenicol #2 and Terramycin	1/4 $8x10^{-2}$
4	"	$2x10^{-9}$	Chloramphenicol #2 and Terramycin	1/4 $8x10^{-2}$
5	"			
6	"			
7	Influenza	$2x10^{-7}$	-----	
8	"	$2x10^{-5}$	Chloramphenicol #2 and Terramycin	1/4 $8x10^{-2}$
9	"	$2x10^{-6}$	Chloramphenicol #2 and Terramycin	1/4 $8x10^{-2}$
10	"	$2x10^{-7}$	Chloramphenicol #2 and Terramycin	1/4 $8x10^{-2}$
11	"	$2x10^{-5}$	Chloramphenicol #2 and Terramycin	1/4 $8x10^{-2}$
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x	x	x	x	x	x			
3	x	x	x	x	x	x	x	x	x			
4	x	x	x	x	x	x	x	x				
5												
6												
7	x	x	x	x	x	x	x	x				
8	x	x	x	x	x	x						
9	x	x	x	x	x	x	x	x	x			
10	x	x	x	x	x	x	x					
11	x	x	x	x	x	x	x					
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination

Table 22

EXPERIMENT 22 *-- The Effect of Chloramphenicol #3 and Dihydrostreptomycin, used Singly and in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-8}$	------	
2	"	$2x10^{-8}$	Chloramphenicol #3	1/4
3	"	$2x10^{-8}$	Dihydrostreptomycin	$4x10^{-2}$
4	"	$2x10^{-8}$	Combination	
5	"			
6	"			
7	Influenza	$2x10^{-6}$	------	
8	"	$2x10^{-6}$	Chloramphenicol #3	1/4
9	"	$2x10^{-6}$	Dihydrostreptomycin	$4x10^{-2}$
10	"	$2x10^{-6}$	Combination	
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x								
3	x	x	x	x	x	x	x					
4	x	x	x	x	x							
5												
6												
7	x	x	x	x	x	x	x	x				
8	x	x										
9	x	x	x	x	x	x	x	x	x			
10	x	x	x									
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

EXPERIMENT 23 * -- The Effect of Dihydrostreptomycin and Terramycin, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-8}$	-----	
2	"	$2x10^{-6}$	Dihydrostreptomycin and Terramycin	$4x10^{-2}$ $8x10^{-2}$
3	"	$2x10^{-7}$	Dihydrostreptomycin and Terramycin	$4x10^{-2}$ $8x10^{-2}$
4	"	$2x10^{-8}$	Dihydrostreptomycin and Terramycin	$4x10^{-2}$ $8x10^{-2}$
5	"			
6	"			
7	Influenza	$2x10^{-7}$	-----	
8	"	$2x10^{-5}$	Dihydrostreptomycin and Terramycin	$4x10^{-2}$ $8x10^{-2}$
9	"	$2x10^{-6}$	Dihydrostreptomycin and Terramycin	$4x10^{-2}$ $8x10^{-2}$
10	"	$2x10^{-7}$	Dihydrostreptomycin and Terramycin	$4x10^{-2}$ $8x10^{-2}$
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x	x							
3	x	x	x	x	x	x	x					
4	x	x	x	x	x	x						
5												
6												
7												
8												
9												
10												
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 24

EXPERIMENT 24 * — The Effect of Penicillin and Terramycin, used in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-8}$	-----	
2	"	$2x10^{-6}$	Penicillin and Terramycin	1/10 $8x10^{-2}$
3	"	$2x10^{-7}$	Penicillin and Terramycin	1/10 $8x10^{-2}$
4	"	$2x10^{-8}$	Penicillin and Terramycin	1/10 $8x10^{-2}$
5	"			
6	"			
7	Influenza	$2x10^{-7}$	-----	
8	"	$2x10^{-5}$	Penicillin and Terramycin	1/10 $8x10^{-2}$
9	"	$2x10^{-6}$	Penicillin and Terramycin	1/10 $8x10^{-2}$
10	"	$2x10^{-7}$	Penicillin and Terramycin	1/10 $8x10^{-2}$
11	"			
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x				
2	x	x	x	x	x	x	x					
3	x	x	x	x	x	x	x	x				
4	x	x	x	x	x	x	x					
5												
6												
7	x	x	x	x	x	x	x					
8	x	x	x	x	x	x	x	x				
9	x	x	x	x	x	x	x	x				
10	x	x	x	x	x	x	x	x				
11												
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 25

EXPERIMENT 25* — The Effect of Penicillin and Terramycin, used Singly and in Combination, on Newcastle Disease Virus and Influenza Virus in Chick Embryos

GROUP	VIRUS	CONC.	DRUG	CONC.
1	Newcastle	$2x10^{-6}$	------	
2	"	$2x10^{-6}$	Penicillin	1/10
3	"	$2x10^{-6}$	Terramycin	$8x10^{-2}$
4	"	$2x10^{-6}$	Combination (equal amounts of penicillin & terramycin)	
5	"	$2x10^{-7}$	------	
6	"			
7	Influenza	$2x10^{-6}$	------	
8	"	$2x10^{-6}$	Penicillin	1/10
9	"	$2x10^{-6}$	Terramycin	$8x10^{-2}$
10	"	$2x10^{-6}$	Combination (equal amounts of penicillin & terramycin)	
11	"	$2x10^{-7}$	------	
12	"			

	DILUTION OF ALLANTOIC FLUID											
GROUP	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384
1	x	x	x	x	x	x	x	x	x			
2	x	x	x	x	x	x	x	x	x	x		
3	x	x	x	x	x	x	x	x	x			
4	x	x	x	x	x	x	x	x	x			
5	x	x	x	x	x	x	x	x	x			
6												
7	x	x	x	x	x	x	x	x	x	x		
8	x	x	x	x	x	x	x	x	x			
9	x	x	x	x	x	x	x	x				
10	x	x	x	x	x	x	x	x	x	x		
11	x	x	x	x	x	x	x	x	x			
12												

*Blank spaces indicate a negative to the last titer.
x indicates a positive hemagglutination.

Table 26.--The Effect of Aureomycin and Dihydrostreptomycin, used Singly and in Combination, on Influenza Virus in Mice

GROUP	DRUG	CONC.	DAYS AFTER INOCULATION			SACRIFICED 8th DAY
			6	7	8	
I	Aureomycin	$2x10^{-3}$	4, 1	----	4*	0
II	Dihydrostreptomycin	$2x10^{-2}$	---	4, 4	---	2
III	Combination	---	4	---	---	0, 0
IV	Control	---	---	4	---	2, 4

*Each number indicates the degree of infection in one mouse on a scale of 0 to 4

Table 27.--The Effect of Aureomycin, Dihydrostreptomycin, Neomycin, and Terramycin, used Singly and in Combination, on Influenza Virus in Mice

GROUP	DRUG	CONC.	DAYS AFTER INOCULATION			SACRIFICED
			2	3	4	5th DAY
I	Aureomycin	$2x10^{-3}$	----	----	4 *	1, 1, 2, 2
II	Dihydro-streptomycin	$2x10^{-2}$	----	----	4	1, 1, 1, 1
III	Neomycin	$1x10^{-2}$	4	4	4	1, 3
IV	Terramycin	$4x10^{-2}$	----	----	4, 4	2, 3, 1
V	Aureomycin and Dihydrostrepto-mycin		----	4	3, 4	1
VI	Aureomycin and Neomycin		----	----	----	0, 1, 2, 3, 4
VII	Aureomycin and Terramycin		----	----	4	1, 1, 3, 3
VIII	Dihydrostrepto-mycin and Neomycin		----	4	4	1, 1, 2
IX	Dihydrostrepto-mycin and Terramycin		----	----	4, 4, 4, 4	0
X	Neomycin and Terramycin		----	----	4, 4	1, 1, 2
XI	Control		----	----	4	2, 2, 3, 3

*Each number indicates the degree of infection in one mouse on a scale of 0 to 4

Table 28.—The Effect of Aureomycin and Terramycin, used Singly and in Combination, on Influenza Virus in Mice

GROUP	DRUG	CONC.	DAYS AFTER INOCULATION			SACRIFICED
			2	3	4	4th DAY
I	Aureomycin	2×10^{-3}	0	——	4 *	0
II	Terramycin	4×10^{-2}	——	4, 3	——	3, 3
III	Combination		——	4	4, 4	0
IV	Control		——	1, 2	4	——

*Each number indicates the degree of infection in one mouse on a scale of 0 to 4

Table 29.—The Effect of Penicillin and Terramycin, used Singly and in Combination, on Influenza Virus in Mice

GROUP	DRUG	CONC.	DAYS AFTER INOCULATION 2	3	4	SACRIFICED 3rd DAY
I	Penicillin	$1x10^{-2}$	4, 4, 4	4	4 *	1, 2
II	Terramycin	$4x10^{-2}$	4	———	4	2, 2, 3, 3
III	Combination		4, 4	4	4, 4, 4, 4	———
IV	Control		4, 4	———	4, 4, 4, 4.	———

*Each number indicates the degree of infection in one mouse on a scale of 0 to 4.

Table 30--The Effect of Aureomycin and Chloramphenicol #2, used Singly and in Combination, on Influenza Virus in Mice

GROUP	DRUG	CONC.	DAYS AFTER INOCULATION			SACRIFICED
			2	3	4	5th DAY
I	Aureomycin	2×10^{-3}	----	----	3, 4,* 4	2, 2
II	Chloramphenicol #2	1/4	----	4	4	2, 2, 3
III	Combination		----	----	3, 4, 4	0, 3
IV	Control		4, 4	4	4	0

*Each number indictes the degree of infection in one mouse on a scale of 0 to 4+

Table 31.—The Effect of Aureomycin, Chloramphenicol #4, and Terramycin, used Singly and in Combination, on Influenza Virus in Mice

GROUP	DRUG	CONC.	DAYS AFTER INOCULATION					SACRIFICED
			3	4	5	6	7	8th DAY
I	Aureomycin	$2x10^{-3}$	----	----	4,3	4,4,4	3*	----
II	Chloramphenicol #4	1/10	0,0,0	----	----	3,3,3	----	----
III	Terramycin	$2x10^{-2}$	----	----	4	4	3,4,4,4	----
IV	Aureomycin and Chloramphenicol #4		----	----	2,3,3	3	4	2
V	Aureomycin and Terramycin		----	----	4,4	4	4,4	2
VI	Chloramphenicol #4 and Terramycin		2,3	1	----	----	----	2,2,1
VII	Control		----	----	4,4	----	4	3,3,3

*Each number indicates the degree of infection in one mouse on a scale of 0 to 4.

Table 32.--The Effect of Aureomycin, Neomycin and Terramycin, used Singly and in Combination, on Influenza Virus in Mice

GROUP	DRUG	CONC.	DAYS AFTER INOCULATION				SACRIFICED
			2	3	4	5	5th DAY
I	Aureomycin	$2x10^{-3}$	---	3,4, 4	4,4,	4*	-----
II	Neomycin	$1x10^{-2}$	---	4,4	4,4	---	1,3
III	Terramycin	$4x10^{-2}$	---	4,4	4,4, 4	4	---
IV	Aureomycin and Neomycin		4	3,4, 4,4	---	---	3
V	Aureomycin and Terramycin		---	---	4,4, 4,4	4	---
VI	Neomycin and Terramycin		2,3	3,4, 4	---	4	---
VII	Control		---	4,4, 4	4,4, 4	---	---

*Each number indicates the degree of infection in one mouse on a scale of 0 to 4.

Table 33.--The Effect of Dihydrostreptomycin, Penicillin and Terramycin, used Singly and in Combination, on Influenza Virus in Mice

GROUP	DRUG	CONC.	DAYS AFTER INOCULATION				SACRIFICED
			5	6	7	8	8th DAY
I	Dihydro-streptomycin	$2x10^{-2}$	---	---	---	4*	1,2 2,3
II	Penicillin	$1x10^{-2}$	---	---	---	3,4	2,2 3
III	Terramycin	$4x10^{-2}$	---	4,4	4	---	3,3
IV	Dihydro-streptomycin and Penicillin		4	4	4	---	2,2
V	Dihydro-streptomycin and Terramycin		---	4	4	4	1,2
VI	Penicillin and Terramycin		---	3	4,4	4	3
VII	Control		---	4,4	4	---	1

*Each number represents the degree of infection in one mouse on a scale of 0 to 4+

TABLE 34.—A Summary of Tables 1-25

DRUG	NO INHIB. WHEN CONC. OF VIRUSES WERE:		SLIGHT INHIB. WHEN CONC. OF VIRUSES WERE:		MOD. TO COMPLETE INHIB. WHEN CONC. OF VIRUSES WERE:	
	INFL.	NDV	INFL.	NDV	INFL.	NDV
Aureomycin	1/2-1/10	1/2-10^{-6}	10^{-3}-10^{-6}			
Chloramphenicol #1	1/2	1/2				
" #2					10^{-6}	10^{-6}
#3					10^{-5}-10^{-7}	10^{-7}-10^{-9}
Dihydrostreptomycin	10^{-8}	10^{-8}				
Neomycin	1/2	1/2				
Penicillin	10^{-6}	10^{-6}				
Terramycin		10^{-6}	10^{-6}			
Aureomycin and Chloramphenicol #2					10^{-5}-10^{-7}	10^{-7}-10^{-9}
Aureomycin and Dihydrostreptomycin	1/2	1/2				
Aureomycin and Neomycin	10^{-5}-10^{-6}	10^{-7}	10^{-7}			10^{-8}-10^{-9}
Aureomycin and Penicillin	10^{-5}-10^{-7}	10^{-5}-10^{-7}				
Aureomycin and Terramycin	1/2-10^{-7}	1/2-10^{-7}				
Chloramphenicol #1 and Streptomycin	1/2	1/2				
Chloramphenicol #2 and Terramycin			10^{-6}	10^{-8}		
Chloramphenicol #3 & Dihydrostreptomycin					10^{-8}	10^{-8}
Dihydrostreptomycin and Terramycin				10^{-6}-10^{-8}		
Neomycin and Terramycin			1/2	1/2		
Penicillin and Terramycin	10^{-5}-10^{-7}	10^{-7}-10^{-9}				

TABLE 35.--A Summary of Tables 26-33 in Terms of the Infectivity Average

DRUG	INFECTIVITY AVERAGE
Aureomycin	3*
Chloramphenicol #2	3
" #4	3
Dihydrostreptomycin	2.3
Neomycin	3.3
Penicillin	3
Terramycin	3.4
Aureomycin and Chloramphenicol #2	2.8
Aureomycin and Dihydrostreptomycin	2.3
Aureomycin and Neomycin	3
Aureomycin and Terramycin	3
Dihydrostreptomycin and Penicillin	3
" and Terramycin	3
" and Neomycin	2.4
Neomycin and Terramycin	3
Terramycin and Penicillin	3.8
Controls	3.5

*Each figure represents the average of approximately 5-30 mice

DISCUSSION

The ineffectiveness of the individual use of penicillin, streptomycin, neomycin and aureomycin in inhibiting a strain of the influenza A virus and Newcastle disease virus in chick embryos and mice is in accord with the findings of Hallaver and Faust (1944), Parker and Diefendorf (1944), Florman et al. (1946), Cutting et al. (1947), Lowell and Buckingham (1946), McKee and Hale (1947), Felsenfeld et al. (1950) and Kass, Lundgren and Finland (1950). The failure of terramycin to inhibit the growth of influenza virus in mice confirms the work of Kass, Lundgren and Finland (1950), Quigland and Francis (1950) and Kass, Barnes and Finland (1950). However, slight inhibition of the influenza virus by this drug was obtained in chick embryos. Similar results were reported by Kass, Barnes and Finland (1950) and by Vinson and Walsh (1950) when the amount of terramycin used was approximately equal to that tried in the present experiments (two mg. for each egg). When higher doses of terramycin were tried by these workers, a greater inhibition resulted. Quigland and Francis (1950) obtained no inhibition of influenza A virus in chick embryos with terramycin.

Three types of chloramphenicol solutions were tested in chick embryos: #1 in which saline was the solvent, #2 in which the drug was dissolved in propylene glycol, and #3 in which acetyl dimethylamine was the vehicle. The lack of inhibition with chloramphenicol #1 confirms the earlier work of Smith et al. (1948). McLean (1949) reported the same results but failed to state the solvent used. Chloramphenicol #2 and #3, however, gave almost complete inhibition of both viruses.

It is possible that the solvents may enable the chloramphenicol to act more efficiently. Due to the relative insolubility of the chloramphenicol in aqueous solutions, it is possible that in saline solution it may not become distributed throughout the area of infection or penetrate the tissues. Effective solvents such as propylene glycol or acetyl dimethylamine may allow these latter actions to occur and thus permit the drug to act to its full capacity. The possibility also exists that the solvent itself could be the inhibitory factor. However, the solvent was probably not present in large enough quantities to be active, as indicated by a propylene glycol control.

The Newcastle disease virus was found to be present only in the spleen and brain tissues of infected chicks and not in the lung or liver tissues. Hofstad (1950) found it to be present in spleen and lung tissues. The difference could be due to the fact that too few experiments were performed in the present work to be conclusive.

The purpose of this series of experiments was to obtain a general overall picture of the individual and combined inhibitory activity of various antibiotics on the viruses and to determine if antagonism or synergism between the antibiotics themselves could be demonstrated. The results presented, however, must be accepted as suggestive rather than conclusive since only a small number of experiments were made with each drug or drug combinations.

Virus growth in the experimental animal is dependant upon (a) concentration, (b) virulence and host adaptability and (c) the age of the experimental animal. Various concentrations of virus were used throughout the investigation in order to see if the amount of virus growth

had any relation to drug inhibition. In some cases it was found that if the initial inoculum was small enough, the virus could be somewhat inhibited by an antibiotic.

The lyophilized form of the influenza virus was passed through mice and the Newcastle disease virus passed through chicks several times before using the viruses in the actual experiments in order to increase their virulence and host adaptability. There is some evidence that the influenza virus can grow in the lungs of mice without producing characteristic lesions (Hirst, 1947), but since the latter are essential criteria in experiments of this type, passage through mice was repeated until the typical symptoms and pathology of the lungs were established.

Kalter (1949a) found that susceptibility or resistance of mice to the influenza virus varied with the age of the animals. Therefore, only young animals were used.

To obviate the error that might result if the chick embryo was dead, the eggs were candled before inoculation. The state of the embryo itself was also noted when the allantoic fluid was harvested. If any signs of embryo deterioration were observed, the eggs were not used or the allantoic fluid was discarded. It was shown by Beveridge and Burnet [illegible] (1946) that embryos were seldom killed by allantoic inoculation.

The hemagglutination test in itself is a highly accurate test for determining the presence of both Newcastle disease and influenza viruses (Miller and Stanley, 1944). However, Florman (1946) and Van Rooyen and Rhodes (1948) states that there are certain factors such as the presence of amorphous ureates, yolk material and profuse bacterial contamination,

which may give a false positive test. To avoid these hazards, all cloudy allantoic fluid samples were discarded.

It was possible that mutual contamination between Newcastle disease and influenza viruses could have occurred during the experiment. The viruses are indistinguishable in the chick embryos, but can be distinguished by their action on mice and chicks. The latter apparently is the natural host for the Newcastle disease virus. Although it may cause lesions in the lungs of mice similar to that of the influenza virus, (Ginsburg, 1951), it occurs only rarely in this animal and then under special conditions (Kilham, 1950). The influenza virus can be easily transmitted as a respiratory infection in mice. In order, then, to eliminate mutual contamination in the embryos, mouse lungs constituted the inoculum of influenza virus in eggs. The mouse adapted strain could infect chick embryos without much change in hemagglutination or pathogenic properties (Kalter, 1949), although the egg adapted strain is much less infectious for mice (Knight, 1946). For similar reasons the chicks were inoculated at various intervals with Newcastle disease virus from the embryo in order to conclusively establish the presence of the specific virus.

A question arose as to the possible correlation of the results of the hemagglutination test of allantoic fluid and the infectivity test in mice, since the properties of the virus causing hemagglutination appears not to be the same as the ones causing infectivity (Burnett, Beveridge, McEwin and Bouke, 1945; Granoff, Liu and Henle, 1950; Chambers and Henle, 1943; Wang, 1948.) The hemagglutination test seems to measure only the

amount of virus present, but not necessarily its ability to infect. Hirst (1942) however, stated that hemagglutination ability is a good index of infectivity under certain conditions, if the strain used in mice experiments is adapted to them and if in the chick embryo experiments, the allantoic fluid is fresh. Both of these conditions were satisfied in the present experiments.

SUMMARY AND CONCLUSION

The purpose of this investigation was to determine the action of the antibiotics penicillin, streptomycin, dihydrostreptomycin, chloramphenicol, neomycin, aureomycin and terramycin used singly or in various combinations, against the PR8 strain of influenza A virus in chick embryos and mice and against Newcastle disease virus in chick embryos.

Of the individual antibiotics, only chloramphenicol, dissolved in propylene glycol or acetyl dimethylamine demonstrated any inhibitory action against either virus in chick embryos. None of the antibiotics tried singly were active against the influenza A virus in mice.

When the antibiotics were used in combination, only aureomycin and neomycin appeared to be synergistic in chick embryos and this occurred only when the virus was used in low concentrations. Terramycin seemed to inhibit the action of chloramphenicol #2 in chick embryos. None of the combinations of antibiotics tried gave any inhibition of the influenza A virus in mice.

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