STUDIES OF SOME FACTORS TO BE CONSIDERED IN EVALUATING THE EFFECT OF CERTAIN CHEMICAL AGENTS ON NEWCASTLE DISEASE VIRUS

By

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A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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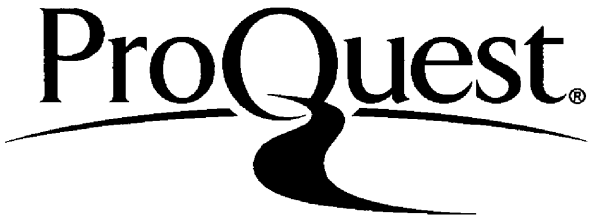

This work is

respectfully dedicated

to

MY FAMILY

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AN ABSTRACT

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Approved 

Studies of the effect of phenol, one per cent, ethyl alcohol, 40 per cent, sodium hydroxide, one per cent; sodium hypochlorite, 440 p.p.m. available chlorine; Roccal, 0.01 per cent; mercuric chloride, 0.01 per cent; and tincture of metaphen, 0.01 per cent, on Newcastle disease virus in proportions of virus:chemical agent of 1:1 and 1:9 at 0 C, 20 C and 37 C for different time intervals indicated a logarithmic decrease in viral activity when undiluted virus-infected allantoic fluid, 0.54 mg nitrogen per ml, $l.d._{50}$ $10^{8.8}$ per 0.1 ml, was employed. Embryonating chicken eggs were used as the indicator host with lethality as the criterion of infectivity of the virus. These findings were substantiated when the chemical agents were tested against different concentrations of the virus at 20 C for 15 minutes.

The initial stage of inactivation of the virus was detected earlier with the 1:9 mixtures than with the 1:1 mixtures. The rate of inactivation for the 1:9 mixtures was greater than that for the 1:1 mixtures with one exception with phenol, one per cent, where the reverse occurred.

Increased temperature augmented the action of ethyl alcohol, sodium hydroxide and sodium hypochlorite. Sodium hydroxide was also efficacious at low temperatures.

These studies showed that the number of infective doses of virus, the ratio of virus to chemical agent, the temperature of exposure, and the period of exposure exert an influence on the evaluation of virucidal tests. Extraneous protein material in the virus preparation may affect the action of oxidizing agents and adsorbing compounds.

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I

INTRODUCTION

Newcastle disease is a highly contagious and fatal virus infection of poultry and of wild birds characterized by pneumonic and neurologic disturbances. Certain animals are susceptible and human infection has been recognized.

Kraneveld (1926) first referred to the disease as one prevalent in poultry in the Netherland East Indies. Later in the same year the disease appeared at Newcastle-on-Tyne in England. Doyle (1927) clearly established the etiology and nature of the disease and proposed the name of Newcastle disease. Since that time the disease has been encountered throughout the world. Due to the widespread distribution of the disease, the name Newcastle disease is obviously unsuited but has been retained to avoid confusion with the plurality of other names (Doyle, 1933). Newcastle disease is now considered to have been present in California as early as 1935, and perhaps earlier, but it was not until 1944 that the virus of a disease first called a "respiratory-nervous disorder" of chickens (Beach, 1941, 1942; Stover, 1942a, 1942b) and later "avian pneumoencephalitis" was recognized as being immunologically identical with the virus of Newcastle disease (Beach, 1944). Newcastle disease virus (NDV) is classified by Holmes (1948) as Tortor furens.

NDV may be found in all internal organs of infected chickens and is excreted from the respiratory and gastro-intestinal tracts (Brandly et al, 1946b). NDV has been detected in the yolk sac of four-day-old chicks,

embryos and infertile eggs laid by hens during the active stages of the infection (DeLay, 1947). Egg transmission does not seem to be a factor in the spread of the disease from hen to chick (Bivins et al, 1950; Hofstad, 1949; Prier et al, 1950). Virus has been recovered from the air in pens of infected chickens (DeLay et al, 1948). Chicks hatched from eggs produced by hens recovered from or vaccinated against the disease may have a naturally acquired passive immunity for three or four weeks. Antibodies may be detected in the yolk of such eggs and chicks (Brandly et al, 1946d; Schmittle, 1950; Schmittle and Millen, 1948).

NDV may be transmitted to persons handling infected birds or working with the virus. In general, the disease is characterized by a superficial, unilateral, acute, granular conjunctivitis with a mucopurulent discharge (Anderson, 1946; Burnet, 1943; Gustafson and Moses, 1951; Hunter et al, 1951; Ingalls and Mahoney, 1949; Keeney and Hunter, 1950; Thompson, 1950). A systemic syndrome of fever, chills, headache, general malaise, mild leucopenia and relative lymphocytosis indicates that the virus is not always limited to the conjunctiva. Virus has been recovered from the blood, nasal and lachrymal secretions, saliva and urine of affected persons (Hunter et al, 1951). Inclusion bodies have not been conclusively demonstrated in the natural host (Jungherr et al, 1946). Recovery is complete in one or two weeks without sequelae. Medication is without effect on the course of the disease.

On the basis of filtration through graded collodion filters, the size of the virus has been estimated to be from 80 mμ to 120 mμ (Burnet and Ferry, 1934). Spherical as well as sperm-shaped particles have been observed by electron microscopy (Bang, 1948c, 1949; Cunha et al, 1947;

Reagan et al, 1948). The virus is spherical in solutions of physiological concentration but increasing hypertonicity produces filamentous and sperm-shaped particles that are to be considered as morphological artifacts (Bang, 1948c, 1949). The head-piece of the sperm-shaped particles is about 70 mμ x 180 mμ (Cunha et al, 1947), 83 x 146 mμ (Bang, 1948c), and the tail-piece about 500 mμ (Cunha et al, 1947). The diameter is from 100 mμ to 125 mμ (Reagan et al, 1948). The virus is a complex of about 67 per cent protein, about 27 per cent lipid and a relatively small amount of nucleic acid, some of which is of the desoxypentose type (Cunha et al, 1947).

The virus passes through all grades of Berkefeld, Mandler and Seitz filters and Chamberland L3 and L5 filters (Beaudette, 1943, 1949b, 1950; Beaudette et al, 1949; Brandly, 1950; Brandly et al, 1946b, Doyle, 1927).

The 50 per cent end point infective unit of NDV contains an average of $10^{-14.72}$ gram of nitrogen, corresponding to $10^{-13.72}$ gram of virus (Cunha et al, 1947). These values suggest an embryo infective unit of 10 particles and verify Bang's (1948a) calculations based on infectivity determinations with four strains of virus.

According to Moses (1948), NDV is more resistant to a basic environment than to an acidic environment. At one week a maximal stability of the virus may be expected within approximately pH 5 to pH 9 (Moses et al, 1947).

NDV possesses the ability to agglutinate red blood cells of chickens and other avian species as well as those of certain mammals (Brandly et al, 1946b; Hanson et al, 1950; U.S.D.A., 1946b; Winslow et al, 1950). This hemagglutinative activity of the virus is inhibited by specifically

immune serum and may be measured quantitatively.

NDV is capable of producing fatal infection of embryonating chicken eggs following injection by any route of inoculation (Bang, 1948a; 1948b; Brandly et al, 1946b; Cunningham, 1952a, 1952b; Hanson et al, 1947; U.S.D.A., 1946a). This characteristic of the virus is utilized as an initial diagnostic criterion for isolation and identification of the virus in tissue specimens from natural outbreaks of the disease. Death of the embryos generally occurs on the third day after inoculation. Dermal petechiation and congestion and hemorrhage of the yolk sac may be observed in dead embryos but there are no characteristic gross pathological alterations of the embryo that can be used for diagnosis (Jungherr et al, 1946). The lethality of NDV is neutralized by specifically immune serum and may be used for a quantitative measurement of the antibody content of the serum (Cunningham, 1951).

The heat stability of the hemagglutinative activity and embryo infectivity of certain strains of the virus at 56 C for 30 minutes is variable. With some strains the hemagglutinative activity is more stable than the embryo infectivity and with others the opposite is found. The heat stability of the hemagglutinative activity of the virus may be used as a genetic marker (Hanson et al, 1949; Durusan, 1949).

A serological relationship of mumps and Newcastle disease has been observed but it is suggested that a diagnosis of Newcastle disease in humans be made with caution, especially in the absence of virus isolation (Jungherr et al, 1949).

The relationship of NDV with the influenza group (Anderson, 1947; Burnet, 1942; Florman, 1948), receptor destruction by viruses of the

mumps-Newcastle disease-influenza group (Hirst, 1950a, 1950b), modification of red blood cells by NDV and use of these cells in serologic studies of infectious mononucleosis and viral hepatitis (Evans, 1950; Kilham, 1950), and NDV hemolysin (Kilham, 1949) have been reported. While these reports indicate certain serologic relationships of the viruses studied and their possible adaptation to diagnosis of human infections, evaluation of the specificity of the reactions must await further investigation.

II

HISTORICAL REVIEW

Many tests of the effect of chemical agents on NDV have been conducted with virus preparations containing varying amounts of tissue elements and extraneous protein material. Mixtures of the virus and chemical were incubated for a certain reaction period and then injected into a suitable host for an indication of the infectivity of the virus. The results have been expressed purely on a qualitative basis as to the ability or inability of the agent to inactivate the virus completely or partially.

Evidence of the infectivity of NDV may be detected by injecting chickens or embryonating chicken eggs with the virus. A measurable reaction such as definite symptoms or mortality may be used as an indication of the activity of the virus. When chickens are used, lethality is the usual criterion of viral activity. If lethality is not the sole criterion, respiratory and nervous manifestations of the disease may require long periods of observation. The greatest limitations to the use of chickens are adequate isolation facilities for prevention of cross infection and a constant supply of suitable birds. Embryonating chicken eggs are the medium of choice because lethality may be used as the criterion of viral infectivity. In addition, the use of eggs has the advantages of ready availability and economy, saving in expense of feed and quarters, minimum danger of cross infection and lack of production of antibodies against the virus injected. Toxic levels of the chemical agent for the indicator host must be previously determined to eliminate the influence of this

factor in interpretation of the results.

Among the problems involved in the evaluation of virucidal agents is that of interpretation of the results obtained in one laboratory in terms of those obtained in another laboratory. Obviously, the difficulty could be largely eliminated by use of a common method in which certain physical factors are standardized. The purpose of the present study is to obtain some information as to the effect of the number of infective doses of the virus, the ratio of virus to chemical agent, the temperature of exposure, and the period of exposure as influencing the evaluation of virucidal tests using embryonating chicken eggs as the indicator host for purposes of standardization. These factors are also to be used in studies of the rate of inactivation of the virus.

The agents selected for study were phenol, one per cent, mercuric chloride, 0.01 per cent, ethyl alcohol, 40 per cent, Roccal (alkyl-dimethyl-benzyl-ammonium chloride) 0.01 per cent, Roman cleanser (sodium hypochlorite, 5.25% by weight), one per cent, and tincture of metaphen (sodium 4-nitro-anhydro-hydroxy-mercurio-orthocresol) 0.01 per cent. Previous studies (Cunningham, 1948) showed that in the above concentrations, these agents were not effective against NDV at three minute reaction periods. In greater concentrations generally employed for disinfection in practice, these agents were effective in inactivating NDV in at least three minutes at room temperature. To study the rate of inactivation of NDV, it was considered feasible to use the above concentrations of the agents as their effect on the virus would be extended over a greater period of time than if greater concentrations were used. This would permit a more accurate evaluation of the rate of inactivation.

Doyle (1927) tested the effect of a number of chemical agents on NDV by mixing equal parts of virus-infected chicken saliva, infective in a 10^{-6} dilution, and allowing the mixtures to react for one hour at room temperature (14-15 C). The mixture was then injected intravenously into susceptible chickens in a dose of one ml. The criterion of infectivity of the virus in the mixtures was death of the chicken. Various dilutions of the chemical agent were employed and the following results from this report show the highest dilutions of the chemical agent in which the virus was either active or inactive:

Agent	Virus active	Virus inactive
Potassium permanganate	1:10,000	1:5,000
Lysol	1:5,000	1:1,000
Izal	1:1,000	1:500
Cresol (Rideal-Walker coefficient 18-20)	1:1,000	1:500
Carbolic acid	1:100	1:20
Mercuric chloride	1:100	
Oil of cloves	1:100	
Sodium salicylate	1:50	1:20
Copper sulphate	1:50	1:20
Hydrogen peroxide (20 vols.)	1:10	1:2

With virus active in a $10^{-5.7}$ dilution (1:500,000) the following results were obtained:

Agent	Virus active	Virus inactive
Antiformin	1:500	1:100
Formalin	1:50	
Acetone	1:5	1:2
Ether		1:5
Methyl alcohol	1:4	1:2
Ethyl alcohol	1:4	1:2
Sodium hydroxide	N/100	N/50
Hydrochloric acid	N/25	

Complete information is lacking as to the procedures employed by Farinas (1930) in his studies of the effect of chemical agents on NDV. Chickens were probably used as the indicator host for virus infectivity. With the exception of the statement that potassium permanganate was tested by oral administration and sodium hydroxide by intravenous inoculation, information is not available as to the potency of the virus, route of administration, amount of inoculum, proportions of virus and chemical agent in the mixtures and temperature of exposure. Clenzal, 2.5 per cent, killed the virus in 30 minutes; one and two per cent formalin killed the virus in 30 minutes, but 0.1 and 0.5 per cent were without effect; one per cent chloroform killed the virus in 30 minutes but 0.1 per cent did not kill in 30 minutes at ice-box temperature; hydrochloric acid, N/1, killed the virus in one hour but N/10 and greater dilutions were ineffective. Attempts to test the action of sodium hydroxide were not successful as a N/2,500 solution by intravenous injection in itself was lethal. Potassium permanganate in dilutions from 1:2,000 to 1:10,000 was sufficient to render the virus innocuous.

Asplin (1949) employed embryonating chicken eggs as the indicator host for studies of the effect of chemical agents on NDV. Undiluted virus-infected allantoic fluid was used. The titer was expressed as the log concentration of minimum lethal doses (m.l.d.) per ml. Information was lacking as to the proportion of virus and agent in the mixtures, age of embryos and amount of inoculum. The mixtures were kept for one hour at the exposure temperature indicated and then stored at refrigerator temperature for the duration of the exposure period. At the end of the exposure period the mixtures were titrated for an indication of the activity

of the virus. Sodium carbonate, 4 per cent, was ineffective against 10^8m.l.d. for one hour and 24 hours at 76 F but at seven days the titer of the virus was reduced from 10^8 to 10^5. Lysol, 2.5 per cent, for one minute at 60 F reduced the infectivity from 10^6 to complete inactivity. A one per cent solution completely inactivated 10^8 m.l.d. in one hour at 75 F but a 0.1 per cent solution reduced the titer from 10^8 to 10^4. A 0.1 per cent solution at 75 F for 24 hours was capable of reduction of the titer from 10^8 to 10^2.

Phenol, 2.5 per cent, at 68 F reduced the titer from 10^7 to 10^2 after exposure for one hour. After 24 hours the virus was completely inactivated. At 68 F a 0.5 per cent solution reduced the titer from 10^7 to greater than 10^5 in 24 hours.

A proprietary hypochlorite disinfectant in 5 per cent solution, the concentration recommended by the manufacturer for disinfection of grossly contaminated material, reduced the titer from 10^7 to one greater than 10^2 at 73 F for one hour. The same concentration at 68 F for three hours completely inactivated 10^6 m.l.d. A one per cent solution at 68 F for one hour was ineffective but at 24 hours the titer was reduced from 10^6 to 10^5.

Potassium permanganate in 0.4 per cent concentration for one hour at 60 F completely inactivated 10^7 m.l.d. but 0.2 per cent under the same conditions reduced the titer from 10^7 to one greater than 10^2. A proprietary permanganate disinfectant at 4.0 per cent concentration, the concentration recommended by the manufacturer for disinfection of grossly contaminated material, had no effect on the virus for one hour at 65 F as shown by no reduction of the titer from 10^5. After 24 hours the titer

was decreased from 10^5 to 10^2. In one per cent solution the titer was reduced from 10^5 to one greater than 10^4.

A commercial coal-tar disinfectant in 2.5 per cent concentration completely inactivated 10^5 m.l.d. in one minute at 60 F. In 1.25 per cent concentration at 75 F for one hour, the concentration recommended by the manufacturer for disinfection of grossly contaminated material, the titer was reduced from 10^7 to 10^2. In 0.5 per cent solution the titer was reduced from 10^7 to 10^3 at one hour at 75 F. Complete inactivation occurred in 24 hours.

Another commercial coal-tar disinfectant in 2 per cent solution, the concentration recommended by the manufacturer for disinfection of grossly contaminated material, reduced the titer from 10^8 to 10^3 within one hour at 67 F and from 10^8 to 10^1 within 24 hours. In one per cent solution at one hour at 67 F the titer was reduced from 10^8 to 10^2 and after 24 hours from 10^8 to 10^1.

The effect of formalin against the virus was found to be influenced by temperature. Virus exposed to 2 per cent formalin was active after one hour at 65 F but inactive in 12 hours. One per cent formalin also inactivated the virus in 12 hours. Concentrations of formalin ranging from 0.2 per cent to 0.025 per cent required from 10 to 90 days to inactivate the virus at 34-35 F. Virus exposed to 0.1 per cent formalin at 98 F was inactivated within six hours.

Tilley and Anderson (1947), Cunningham (1948) and Beamer and Prier (1950) studied the effect of several chemical agents on NDV. The criterion of inactivation of the virus was the survival of embryonating chicken eggs during a definite observation period following inoculation with a mixture of the virus and agent. The procedures used in these tests varied in

several respects, viz., concentration of the agents, proportions of the virus and agent in the mixture, reaction period, subsequent dilution of the mixture prior to inoculation of the embryos, amount of inoculum, number of embryos per test, and the length of the observation period. Notwithstanding these variations, certain comparisons can be made of the effectiveness of the chemical agents commonly employed in these studies. In Table I are presented data from these studies with the chemical agents employed in the present study.

Sodium hydroxide, 2 per cent, inactivated NDV during three and five minute reaction periods. Sodium hydroxide, one per cent, was without effect on NDV for 60 minutes. In 0.1 per cent concentration it was without effect during a three minute reaction period.

Phenol, 4 per cent, partially inactivated the virus, only one of four embryos succumbing to the virus, at five minutes according to Tilley and Anderson. At the 15, 30 and 60 minute periods the virus was completely inactivated. In 3 per cent concentration, phenol completely inactivated the virus in three minutes according to Cunningham. A 2 per cent solution of phenol was without effect during the entire 60 minute period according to Tilley and Anderson. The data presented by Beamer and Prier indicate that at five minutes exposure to phenol, 2 per cent, only three of five embryos were killed. At 30 minutes the virus was completely inactivated. Phenol, one per cent, was nonvirucidal.

Ethyl alcohol, 95 per cent, was virucidal in three minutes and in 70 per cent concentration in three and five minutes. When used in 50 per cent concentration, it was without effect at five minutes but at 30 minutes the virus was almost completely inactivated, one of five embryos being

killed. Forty per cent and 25 per cent alcohol were without effect in three minutes.

Tincture of metaphen, 0.5 per cent, was considered by Cunningham to be virucidal in three minutes but noneffective in 0.005 per cent concentration. Beamer and Prier reported that in the above concentrations the agent failed to suppress viral activity in 30 minutes.

Roccal, 0.1 per cent, inactivated the virus in three and five minutes. At 0.01 per cent, it was ineffective according to Cunningham during the three minute reaction period. Tilley and Anderson's data reveal varying efficacy of 0.1 per cent during the 60 minute reaction period.

Mercuric chloride, 0.1 per cent, was virucidal in three minutes but at 0.01 per cent it was ineffective.

Sodium hypochlorite, 0.0525 per cent was without effect in three minutes but 0.2625 per cent and 1.05 per cent were virucidal according to Cunningham. Tilley and Anderson's data indicate that sodium hypochlorite in 200 and 400 parts per million (p.p.m.) available chlorine, pH 11.4, was without effect for 60 minutes.

In addition to the chemical agents listed in Table I, Beamer and Prier found the following to be apparently effective against NDV in five minutes: liquor cresolis saponatus, 1:400; tincture of iodine, undiluted and 1:100; Lugol's solution, 1:1,000; formalin, 2 per cent; potassium permanganate, 1:100 and 1:1,000; merthiolate, 1:1,000. The following were effective in 30 minutes: formalin, 0.5 per cent and liquid green soap, 1:10.

Agents that had no apparent effect were potassium dichromate, 1:100, merthiolate, 1:100,000 and hydrogen peroxide, 8 per cent.

The following quaternary ammonium compounds were effective in inactivating the virus in five minutes: Zepharin, undiluted; para-tertiary-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride monohydrate, 1:500 and Roccal, 1:100. The following were ineffective at five minutes but were virucidal at 30 minutes: Zepharin, 1:100; para-di-isobutyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride, 1:100 and 1:1,000.

According to Tilley and Anderson, the following agents were virucidal: sodium orthophenylphenate, one per cent at five minutes; para-tertiary-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride monohydrate, 0.1 per cent at five minutes; liquor cresolis saponatus, one per cent, almost complete inactivation at 15 minutes and complete inactivation at 30 minutes.

Calcium hypochlorite, 100 and 200 p.p.m. was without effect in 60 minutes, but at 400 p.p.m., pH 8.25, it was virucidal in five minutes.

Formalin, 4 per cent, failed to inactivate the virus in 30 minutes but was effective in 60 minutes.

Isopropyl alcohol, 50 per cent, ethylene glycol, undiluted, and sodium carbonate, 4 per cent, appeared to be ineffective against the virus.

According to Cunningham, the following agents, other than those listed in Table I, were effective against NDV in three minutes: tincture of Zepharin, 0.1 per cent, Phemerol, 3 per cent, Disilyn, 0.02 per cent, liquor cresolis saponatus, 3 per cent, Lysol, 3 per cent and one per cent, creolin Pearson, 3 per cent and one per cent; and tincture of iodine, 2.5 per cent and one per cent. The following agents did not completely inactivate the virus during the three minute reaction period, but they were

considered as being effective against the virus: Phemerol, one per cent, liquor cresolis saponatus, one per cent; potassium permanganate, 0.1 per cent, and formalin, 10 per cent.

The following agents were without effect on the virus during the three minute reaction period: merthiolate, 0.1 per cent and 0.01 per cent, tincture of Zepharin, 0.001 per cent, potassium permanganate, 0.01 per cent, tincture of iodine, 0.1 per cent and 0.01 per cent, formalin, one per cent and 0.01 per cent; and boric acid, 4 per cent.

While similarities in the trends of the effectiveness of these agents are evident in all three studies, certain irregularities in the procedures must be considered. Different concentrations of the agents were employed. This is not of major importance except when direct comparisons are to be made. In several instances, the same concentration of a given chemical agent was used by all authors. It is evident that the authors attempted to establish facts as to the effectiveness of the agents in concentrations at least those generally employed for disinfection as well as at minimum virucidal concentrations.

Differences in the virus:chemical volumetric ratio are obvious. Tilley and Anderson, and Beamer and Prier used virus and agent in equal parts, whereas a 1:9 ratio was used by Cunningham. These differences would result in a marked variation in the number of virus particles at the time of contact with the chemical agents.

Cunningham and Tilley and Anderson used undiluted virus-infected allantoic fluid but Beamer and Prier used a 10^{-3} dilution of the fluid. The virus used by Cunningham contained 10^{8} infective doses per 0.05 ml. Tilley and Anderson reported that their virus, which was titrated for

each experiment, contained from 10^7 to 10^8 infective doses per 0.1 ml. These viruses had been originally established and propagated in chicken embryos for 15 passages and four passages, respectively. Beamer and Prier's virus which contained 10^{10} infective doses per 0.1 ml had been isolated from a natural outbreak of the disease in chickens and carried for 17 serial passages in duck embryos. Chicken embryos were used for titration and for the tests.

For a quantitative comparison of the three different strains of the virus on a uniform basis of 0.1 ml inoculum per egg, the strain used by Cunningham would have to be considered as containing 2×10^8 or $10^{8.3}$ (200,000,000) infective doses as titration was done with 0.05 ml inoculum. When the virus and chemical were mixed in a ratio of 1:9, the mixture would be considered to have $10^{7.3}$ (20,000,000) infective doses. Some of the mixtures were injected directly into the embryos without further dilution at the end of the reaction period but some were diluted as much as 10^{-3}. The number of virus particles injected into embryos in the various tests ranged from $10^{7.3}$ (20,000,000) to $10^{4.3}$ (20,000). See Table I.

The virus used by Beamer and Prier had an initial infective concentration of 10^{10} (10,000,000,000) doses but a 10^{-3} dilution, which would contain 10^7 (10,000,000) infective doses, was used for mixing with the chemical agents. Since the mixtures were in equal parts, there would be $10^{6.7}$ (5,000,000) doses of virus. Subsequent dilutions ranged from $10^{-3.3}$ (1:2,000) to 10^{-4} (1:10,000) and the number of virus particles injected ranged from $10^{3.4}$ (2,500) to $10^{2.7}$ (500). See Table I.

Tilley and Anderson employed virus containing from 10^7 (10,000,000) to 10^8 (100,000,000) infective doses. When mixed with equal parts of

chemical agent from $10^{6.7}$ (5,000,000) to $10^{7.7}$ (50,000,000) doses were present. Subsequent dilutions ranged from $10^{-1.7}$ (1:50) to $10^{-2.3}$ (1:200) which resulted in inocula containing from $10^{4.4}$ (25,000) to $10^{6.0}$ (1,000,000) virus particles. See Table I.

Summation of these data show that the inocula ranged from $10^{2.7}$ (500) to $10^{7.3}$ (20,000,000) or a maximum possible $10^{4.6}$ differential. See Table I.

The number of eggs employed for each test varied in the three studies. The survival or death of one of the four embryos in Tilley and Anderson's study would result in a differential of 25 per cent, and 20 per cent, one of five embryos in Beamer and Prier's work. With Cunningham's work the differential would be 10 per cent or one of ten embryos.

TABLE I

THE EFFECT OF CERTAIN CHEMICAL AGENTS ON NEWCASTLE DISEASE VIRUS AS DETERMINED BY INFECTIVITY TESTS IN EMBRYONATING CHICKEN EGGS*

Chemical	Conc.	Ratio of virus: chemical	Sub-sequent dilution	Per cent embryo mortality Reaction period Minutes 3	5	15	30	60	Ref.
Ethyl alcohol	95%	1:9		0					(1)
	70%	1:9		0					(1)
		1:1	2500		0		0		(2)
	50%	1:1	2500		100		20		(2)
	40%	1:9		100					(1)
	25%	1:9		100					(1)
Tincture of metaphen	0.5%	1:9	1000	0					(1)
	0.5%	1:1	2000		100		100		(2)
	0.005%	1:9	10	100					(1)
	0.005%	1:1	2000		100		100		(2)
Mercuric chloride	0.1%	1:9	100	0					(1)
	0.01%	1:9	10	100					(1)
Phenol	4%	1:1	100		25	0	0	0	(3)
	3%	1:9	100	0					(1)
	2%	1:1	50		100	100	100	100	(3)
		1:1	2000		60		0		(2)
	1%	1:9	100	0					(1)
Sodium hydroxide	5%	1:9	500	0					(1)
	2%	1:9	500	0					(1)
		1:1	100		0	50	25	25	(3)
		1:1	2500		0		0		(2)
	1%	1:1	50		100	100	100	100	(3)
	0.1%	1:9		100					(1)
Roccal	0.1%	1:9		10					(1)
		1:1	2500		0		0		(2)
	0.1%	1:9		100					(1)
		1:1	50		0	25	0	25	(3)

TABLE I (Continued)

Chemical	Conc.	Ratio of virus: chemical	Sub-sequent dilution	Per cent embryo mortality Reaction period Minutes 3	5	15	30	60	Ref.
Sodium hypochlorite	1.05%	1:9	10	0					(1)
	0.2625%	1:9		0					(1)
	0.0525%	1:9		100					(1)
	Available Cl_2 p.p.m.								
	200	1:1	50		100	100	100	100	(3)
	400	1:1	100		100	100	100	100	(3)

* Data from (1) Cunningham (1948), Beamer and Prier (1950), and (3) Tilley and Anderson (1947).

Eggs used per sample: (1) 10, (2) 5, (3) 4
Infective doses of virus: (1) 10^8 per 0.05 ml, (2) 10^{10} per 0.1 ml (3) 10^7 to 10^8 per 0.1 ml
Inoculum per egg: (1) 0.05 ml, (2) (3) 0.1 ml
Temperature of exposure: (1) (2) room temperature, (3) 20 C

McCulloch (1945) focused attention on the fact that there have been marked differences in the procedures employed in studies of the effect of chemical agents on viruses. He suggested that the following factors should be considered for a critical evaluation of virucidal or bactericidal tests:

1. The number of infective doses of the virus.
2. The ratio of virus-containing material to agent.
3. The temperature of exposure.
4. The period of exposure.
5. The size of the particulate matter containing the virus.
6. The nature of the fluid in which the virus is suspended.
    a. pH
    b. buffer capacity
    c. reducing properties
    d. osmotic index
    e. surface tension

III

MATERIALS AND EXPERIMENTAL PROCEDURES

A strain of NDV (Accession 51-52 308) which had been originally isolated from lung and tracheal material from a natural outbreak of the disease in chickens was used. This virus was established and cultivated through 8 serial passages in the allantoic cavity of embryonating chicken eggs and was capable of killing all embryos by the end of the second day after inoculation. The virus preparation employed consisted of pooled allantoic fluid from embryos dead on the second postinoculation day. Portions of the pooled virus were placed in 30 ml capacity screw cap vials and stored at -30 C. At the time of use, the virus was thawed at room temperature, centrifuged to sediment the yellow, insoluble precipitate formed on freezing and thawing, and the supernatant fluid was transferred by pipette to another vial. The virus-infected supernatant fluid contained 0.54 mg. nitrogen per ml as determined by the macro Kjeldahl method (Association of Official Agricultural Chemists, 1950), and $10^{8.8}$ lethal doses per 0.1 ml as determined by titration in eggs (Cunningham, 1952).

The study was divided into two parts: (1) the effect of the various chemical agents on undiluted virus in proportions of virus: chemical agent of 1:1 and 1:9 at 0 C, 20 C and 37 C during various periods of exposure, and (2) the effect of the chemical agents on serial ten-fold dilutions of virus ranging from undiluted through a 10^{-6} dilution in proportions of virus:chemical agent of 1:9 at 20 C for 15 minutes.

All chemical agents and the virus were at thermal equilibrium at the contact period. The chemical agents, which had been prepared in distilled water, were dispensed for the first part of the study in two ml amounts from two ml serological pipettes for the 1:1 mixture in 13 x 100 mm Pyrex tubes and in 4.5 ml amounts from five ml measuring pipettes for the 1:9 mixture. The virus was then added to the chemical agents from two ml serological pipettes, two ml for the 1:1 mixture and 0.5 ml for the 1:9 mixture. The ingredients were thoroughly mixed by aspirating and expelling the mixture 20 times with the pipette. The samples tested at 0 C were placed in crushed ice in a "Thermos" laboratory vessel. An electric, thermostatically controlled water bath was used for the tests at 20 C and 37 C. The level of the water in the baths was above the level of the virus-chemical mixture in the tubes to insure thermal equilibrium throughout the reaction period.

For the second part of the study, serial ten-fold dilutions of the virus through the 10^{-6} dilution were prepared in distilled water using one ml of virus to nine ml of distilled water. The mixtures were prepared in the same manner as the 1:9 mixtures previously described for the first part of the study.

At the end of each reaction period, 0.1 ml of the mixture was transferred with a one ml serological pipette to 9.9 ml of distilled water to dilute the chemical agent to a concentration nontoxic for the embryo. The ingredients were mixed by aspirating and expelling the mixture with the pipette 20 times. Some of the agents in the concentrations employed could be injected directly into eggs while others required further dilution.

Previous studies (Cunningham, 1948) had shown that the following dilutions were sufficient to render the agents nontoxic for embryos:

Agent	Concentration	Dilution
Tincture of metaphen	0.5%	10
Ethyl alcohol	40%	
Mercuric chloride	0.01%	10
Roccal	0.1%	
Sodium hypochlorite	0.0525%	
Phenol	1%	100
Sodium hydroxide	1%	100

These dilutions cover a 100-fold range and for uniformity of inoculum a 10^{-2} dilution was made prior to injection of embryos.

Ten eggs were used for each test at the various reaction periods. Injection was via the allantoic cavity with 0.1 ml inoculum per egg. All incubation of eggs was at 99-99.5 F in an electric, forced-draft incubator (Jamesway Model 252) in which the eggs were automatically turned every two hours. The postinoculation incubation period was seven days. Eggs were candled daily. Embryo mortality during the first 24 hours was considered to be due to nonspecific causes and these eggs were not included in the final results.

Titration of the virus for the first part of the study was accomplished by preparing serial ten-fold dilutions of the virus using nutrient broth as the diluent (0.5 ml virus: 4.5 ml diluent). Five eggs were employed per dilution and each was inoculated with 0.1 ml of the respective dilutions via the allantoic cavity (Cunningham, 1952). The eggs were incubated for seven days following inoculation. Mortality rates were used in computing the titer which was expressed as the lethal dose $_{50}$ (l.d.$_{50}$) according to the method of Reed and Muench (1938). The l.d.$_{50}$ was calculated to the centile and rounded off to the decile. The number of

lethal doses of virus was considered to be the antilog of the reciprocal of the $l.d._{50}$.

Titration of the virus for the second part of the study was accomplished by using the virus dilutions prepared for mixture with the chemical agents. Ten eggs were used per dilution. The procedures and calculation of the $l.d._{50}$ were the same for both parts of the study.

IV

RESULTS

The results of the tests of the infectivity of NDV in mixtures of certain chemical agents in proportions of virus:chemical agent of 1:1 and 1:9 at 0 C, 20 C and 37 C at different time intervals are summarized for the first part of the study in Table II. The criterion of viral infectivity was embryo mortality. Death of all embryos was recorded as 100 per cent, indicating that the virus was not affected by the chemical agent. Survival of all embryos was recorded as 0 per cent, indicating that the chemical agent completely inactivated the virus.

For one of the tests the data best fit (1) a regression line, $Y = a + bX$, and for the other tests (2) a logarithmic curve, $\log Y = a + bX$, as computed from the following respective equations:

(1)
$$Na + \Sigma bX = \Sigma Y$$
$$a \Sigma X + b \Sigma X^2 = \Sigma XY$$

(2)
$$Na + \Sigma bX = \Sigma \log Y$$
$$a \Sigma X + b \Sigma X^2 = \Sigma X \log Y$$

Effect of Chemical Agents on Undiluted Newcastle Disease Virus

In all tests in the first part of the study where the effect of the various chemicals on undiluted virus was investigated, it was assumed that the virus in the mixtures was capable of killing all embryos immediately following contact of the virus with the chemical agents. The data for some tests showed that the virus was capable of killing all embryos throughout the entire series of reaction periods. In some tests, some degree of

inactivation of the virus occurred. For an indication of the rate of inactivation of the virus in the latter tests, the last period at which 100 per cent mortality occurred was given the value 0.0X. The data were used throughout the first period at which inactivation was complete (0.0 per cent mortality) or the terminal reaction period if complete inactivation had not occurred prior to this time.

At 0 C, inoculum from the mixture of equal parts of NDV and phenol, one per cent remained infective for all embryos for 60 minutes. Inoculum containing NDV and phenol in proportions of 1:9 was infective for all embryos for 40 minutes but at 50 minutes the embryo mortality was 90 per cent. At 60 minutes, only 20 per cent of the embryos were killed. These data indicate that for 40 minutes NDV exhibited similar resistance to phenol in proportions of 1:1 and 1:9. During the next ten minutes there was a slight inactivation of the virus in the 1:9 mixture followed by a precipitous decrease in viral activity during the next ten minutes. These data are presented graphically in Figure 1. The straight line regression equation $Y = 110 + (-4.0)X$ best fits the data for the 1:9 mixture during the 40 to 60 minute periods. For the mixture of equal parts, the equation $\log Y = 2.0 + 0.0X$ fits the data.

When NDV and phenol were mixed in equal portions at 20 C, the virus killed all embryos in 30 minutes. During the next ten minutes the infectivity of the virus was markedly reduced so that at 40 minutes only ten per cent of the embryos were killed. At 50 minutes, all embryos survived but ten per cent were killed at the 60 minute period. Inactivation of the virus in the 1:9 mixture occurred at a rapid rate. Only 60 per cent of the embryos were killed ten minutes after contact of NDV with the phenol.

TABLE II

PER CENT EMBRYO MORTALITY FOLLOWING INOCULATION WITH MIXTURES OF NEWCASTLE DISEASE VIRUS AND CERTAIN CHEMICAL AGENTS

Reaction period - mins.	Temperature: 0 C, Proportion of virus:chemical agent 1:1	0 C 1:9	20 C 1:1	20 C 1:9	37 C 1:1	37 C 1:9	Virus l.d.50
			Phenol, 1%, pH 6.4				
10	100	100	100	60	100	-	$10^{8.8}$
20	100	100	100	10	100	50	
30	100	100	100	10	100	20	
40	100	100	10	0	100	-	
50	100	90	0	0	-	-	
60	100	20	10	10	-	-	
			Ethyl alcohol, 40%, pH 6.9				
10	100	100	100	88.8[a]	100	37.5[b]	
20	100	100	100	88.8[a]	100	10	
30	100	100	100	60	100	20	
40	100	100	100	80	100	0	
50	100	100	100	60	60	0	
60	100	100	100	70	-	-	
			Sodium hydroxide, 1%, pH 13.0				
15	90	11.1	30	0	20	-	
30	90	10	30	0	0	-	
45	50	10	20	0	20	-	
60	40	10	30	0	10	-	
75	30	20	40	0	0	-	
90	60	10	20	0	0	-	
105	10	10	30	0	0	-	
120	30	20	0	0	0	-	
			Sodium hypochlorite, 440 p.p.m. available chlorine, pH 10.9				
15	100	10	100	22.2[c]	100	11.1[e]	
30	100	0	100	10	100	0	
45	100	10	100	0	90	0	
60	100	10	100	0	55.5[d]	11.1[e]	
75	100	0	100	0	40	0	
90	100	0	100	10	20	20	
105	100	0	100	0	30	10	
120	100	10	100	0	10	-	

TABLE II (Continued)

Reaction period - mins.	Temperature 0 C Proportion of virus:chemical agent 1:1	1:9	20 C 1:1	1:9	37 C 1:1	1:9
			Roccal, 0.01%, pH 6.1			
15	100	100	100	100	100	0
30	100	100	100	100	100	0
45	100	100	100	100	100	10
60	100	100	100	100	90	0
75	100	100	100	100	100	10
90	100	100	100	100	80	10
			Mercuric chloride, 0.01%, pH 3.5			
15	100	100	100	100	100	100
30	100	100	100	100	100	100
45	100	100	100	100	100	100
60	100	100	100	100	100	100
75	100	100	100	100	100	100
90	100	100	100	100	100	100
105	100	100	100	100	100	100
120	100	100	100	100	100	100
			Tincture of metaphen, 0.01%, pH 8.4			
15	100	100	100	100	100	100
30	100	100	100	100	100	100
45	100	100	100	100	100	100
60	100	100	100	100	100	100
75	100	100	100	100	100	100
90	100	100	100	100	100	100
105	100	100	100	100	100	100
120	100	100	100	100	100	100

a One embryo died from nonspecific causes. Per cent calculated from 8/9 mortality rate

b Two embryos died from nonspecific causes. Per cent calculated from 3/8 mortality rate.

c One embryo died from nonspecific causes. Per cent calculated from 2/9 mortality rate.

d One embryo died from nonspecific causes. Per cent calculated from 5/9 mortality rate.

e One embryo died from nonspecific causes. Per cent calculated from 1/9 mortality rate.

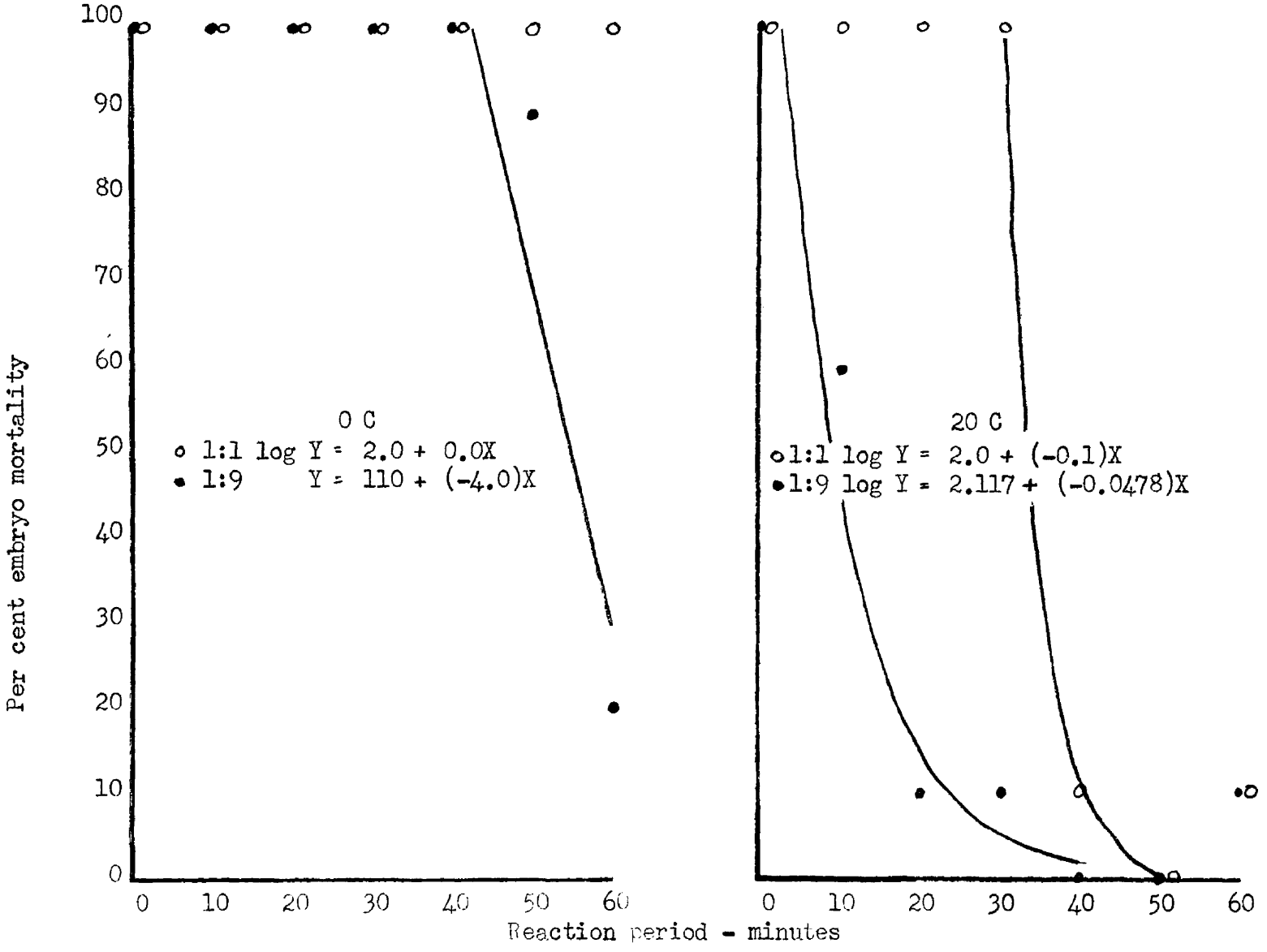

Figure 1. Effect of phenol (1%), pH 6.4, on Newcastle disease virus, l.d.$_{50}$ $10^{8.8}$, in proportions of virus:phenol of 1:1 and 1:9 at 0 C and 20 C.

At the 20 and 30 minute intervals, the mortality was ten per cent. None of the embryos died at the 40 and 50 minute periods but ten per cent were killed at the terminal 60 minute period. The mortality at the terminal period for both mixtures may possibly have been due to nonspecific causes rather than a true reflection of viral activity. These data indicate that at 20 C, inactivation of NDV occurred sooner when virus and phenol were in mixtures of 1:9 than in 1:1 but complete inactivation of the virus in the mixtures differed only by ten minutes. In both instances, as shown in Figure 1, logarithmic curves fit the data reasonably well. The curve for the mixture of equal parts is $\log Y = 2.0 + (-0.1)X$ and for the 1:9 mixture $\log Y = 2.117 + (-0.0478)X$. These curves show that while inactivation occurred sooner in the 1:9 mixture than in the 1:1 mixture, the rate of reaction was greater in the 1:1 mixture when the coefficients of regression are compared.

At 37 C, NDV activity in the mixture of equal parts was sufficient to kill all embryos during the 40 minute reaction period, and the equation $\log Y = 2.0 + 0.0X$ fits the data. Figure 2. Inoculum from the 1:9 mixture killed 50 per cent of the embryos at the 20 minute interval and 20 per cent at the 30 minute period, the only two periods at which samplings were made. The equation $Y = 2.03 + (-0.022)X$ fits these data. The data and equations show that the infectivity of the virus was reduced more rapidly in the 1:9 mixture than in the 1:1 mixture at 37 C.

Summation of the effect of phenol on NDV shows that inactivation of the virus occurs at an earlier period in mixtures of 1:9 than in mixtures of equal parts in the three experiments at 0 C, 20 C and 37 C. At 20 C, the rate of inactivation for the 1:1 mixture was greater than for the 1:9

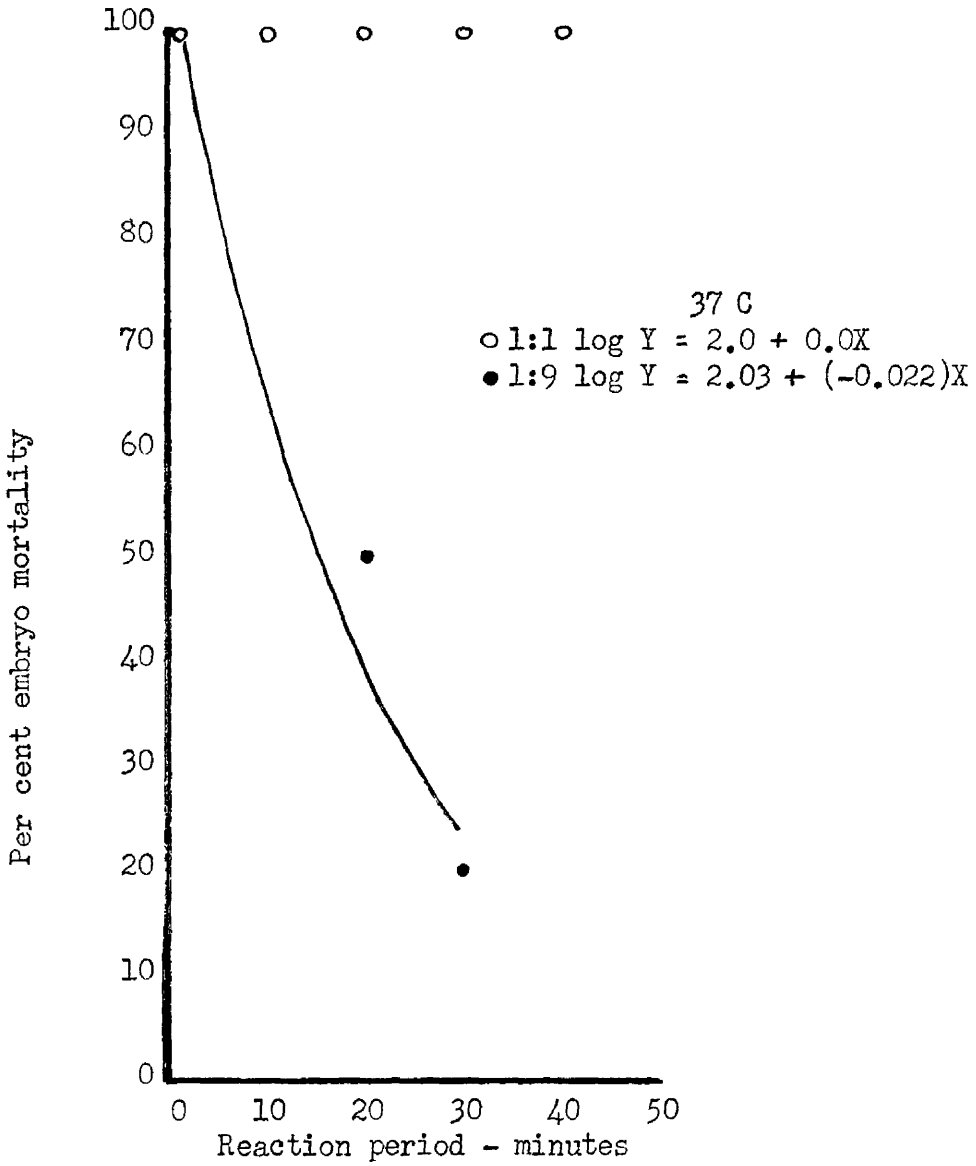

Figure 2. Effect of phenol (1%), pH 6.4, on Newcastle disease virus, $l.d._{50}$ $10^{8.8}$, in proportions of virus:phenol of 1:1 and 1:9 at 37 C.

mixture but occurred at a later time. Comparison of the data for the different temperatures shows that inactivation occurred more rapidly at 20 C than at 0 C.

When eggs were inoculated with mixtures of virus:ethyl alcohol, 40 per cent, in proportions of 1:1 and 1:9, all embryos died throughout the 60 minute period at 0 C. Figure 3. When similar mixtures were exposed at 20 C for 60 minutes, the virus was sufficiently active in the 1:1 mixture to kill all embryos. The equation $\log Y = 2.0 + 0.0X$ fits these data for 0 C and 20 C. Inoculum from the 1:9 mixture killed 88.8 per cent of the embryos (eight of nine embryos) at the ten and 20 minute periods. During the 30 to 60 minute interval, the mortality ranged from 60 to 80 per cent; 60 per cent at 30 minutes, 80 per cent at 40 minutes, 60 per cent at 50 minutes and 70 per cent at 60 minutes. The equation $\log Y = 1.98 + (-0.003)X$ best fits these data. At 37 C the mixture of equal parts of virus and alcohol killed all embryos in 40 minutes, but at 50 minutes only 60 per cent of the embryos were killed. Figure 4. The equation $\log Y = 2.0 + (-0.02)X$ fits these data. The 1:9 mixture killed 37.5 per cent of the embryos (three of eight embryos) at the ten minute interval, ten per cent at 20 minutes and 20 per cent at 30 minutes. Inoculum after 40 and 50 minutes exposure was innocuous. The curve $\log Y = 2.035 + (-0.043)X$ fits these data.

These results show that the effect of ethyl alcohol, 40 per cent, NDV in proportions of 1:1 and 1:9 at 0 C for 60 minutes could not be detected. At 20 C and 37 C, inactivation was more rapid in the 1:9 mixtures than in the 1:1 mixtures. Thermal influence was evident in that

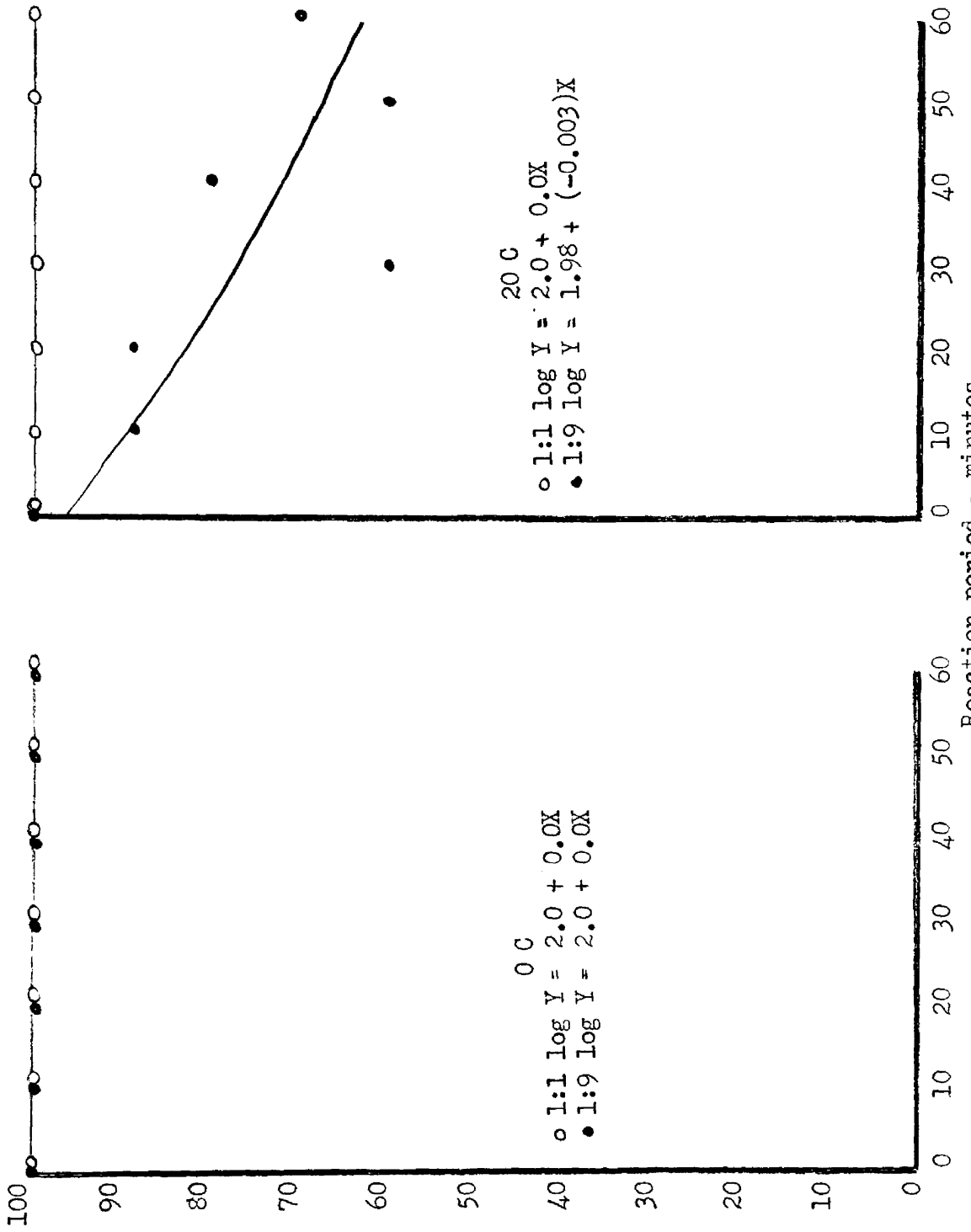

Figure 3. Effect of ethyl alcohol (40%), pH 6.9, on Newcastle disease virus, l.d.$_{50}$ $10^{8.8}$, in proportions of virus:alcohol of 1:1 and 1:9 at 0 C and 20 C.

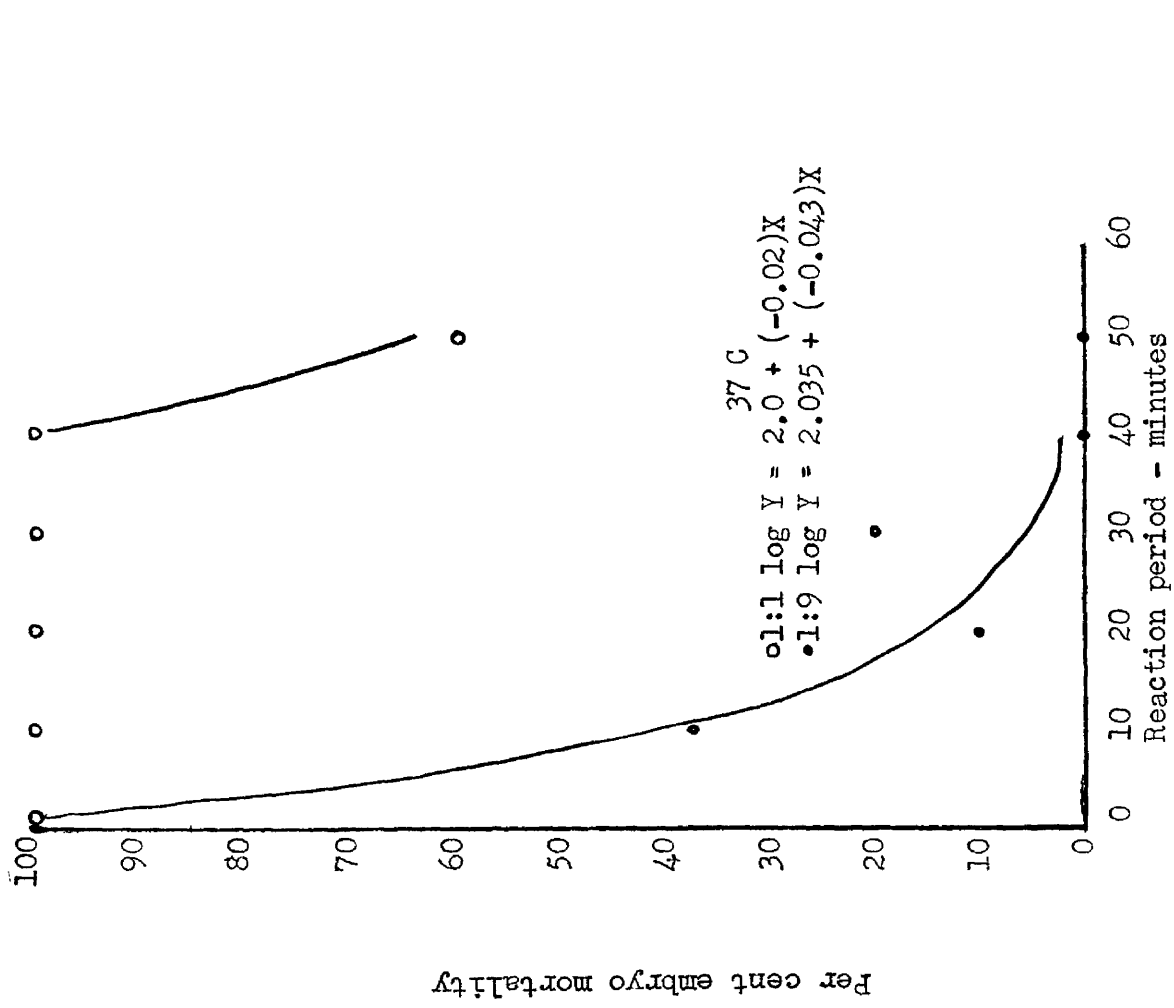

Figure 4. Effect of ethyl alcohol (40%), pH 6.9, on Newcastle disease virus, $1.d._{50}$ $10^{8.8}$, in proportions of virus:alcohol of 1:1 and 1:9 at 37 C.

the rate of inactivation for the 1:9 mixtures was greater at 37 C than at 20 C. Inactivation of the virus in the mixture of equal parts could be detected after 40 minutes exposure.

Sodium hydroxide, one per cent, was markedly virucidal at 0 C. Figure 5. Inoculum from the mixture of equal parts of virus and sodium hydroxide killed 90 per cent of the embryos at the 15 and 30 minute periods. Fifty per cent were killed 45 minutes after contact, 40 per cent at 60 minutes, 30 per cent at 75 minutes, 60 per cent at 90 minutes and ten and 30 per cent at 105 and 120 minutes, respectively. Observation of the data indicates a logarithmic trend in the rate of inactivation if the results for the 90 and 120 minute periods are not considered as representative of the general trend. In this case, the curve, $\log Y = 2.06 + (-0.0082)$ fits the data. Inoculum from the 1:9 mixture killed only 11.1 per cent of the embryos (one of nine embryos) 15 minutes after contact of the virus with the agent. Ten per cent of the embryos were killed at the 30 to 60 minute periods followed by 20 per cent mortality at 75 minutes, ten per cent at 90 and 105 minutes, and 20 per cent at 120 minutes. The equation $\log Y = 2.002 + (-0.0636)X$ fits these data from the time of contact to the 15 minute interval. With the exception of the 75 and 120 minute periods, all subsequent mortality was ten per cent indicating that there was some slight survival of infectivity of the virus. It is obvious that the rate of inactivation was much more rapid with the 1:9 mixture than with the 1:1 mixture but the infectivity of the virus in both mixtures was the same at the 105 minute interval.

At 20 C the data for the mixture of virus and sodium hydroxide in equal parts present some difficulties in interpretation. At 15 and 30

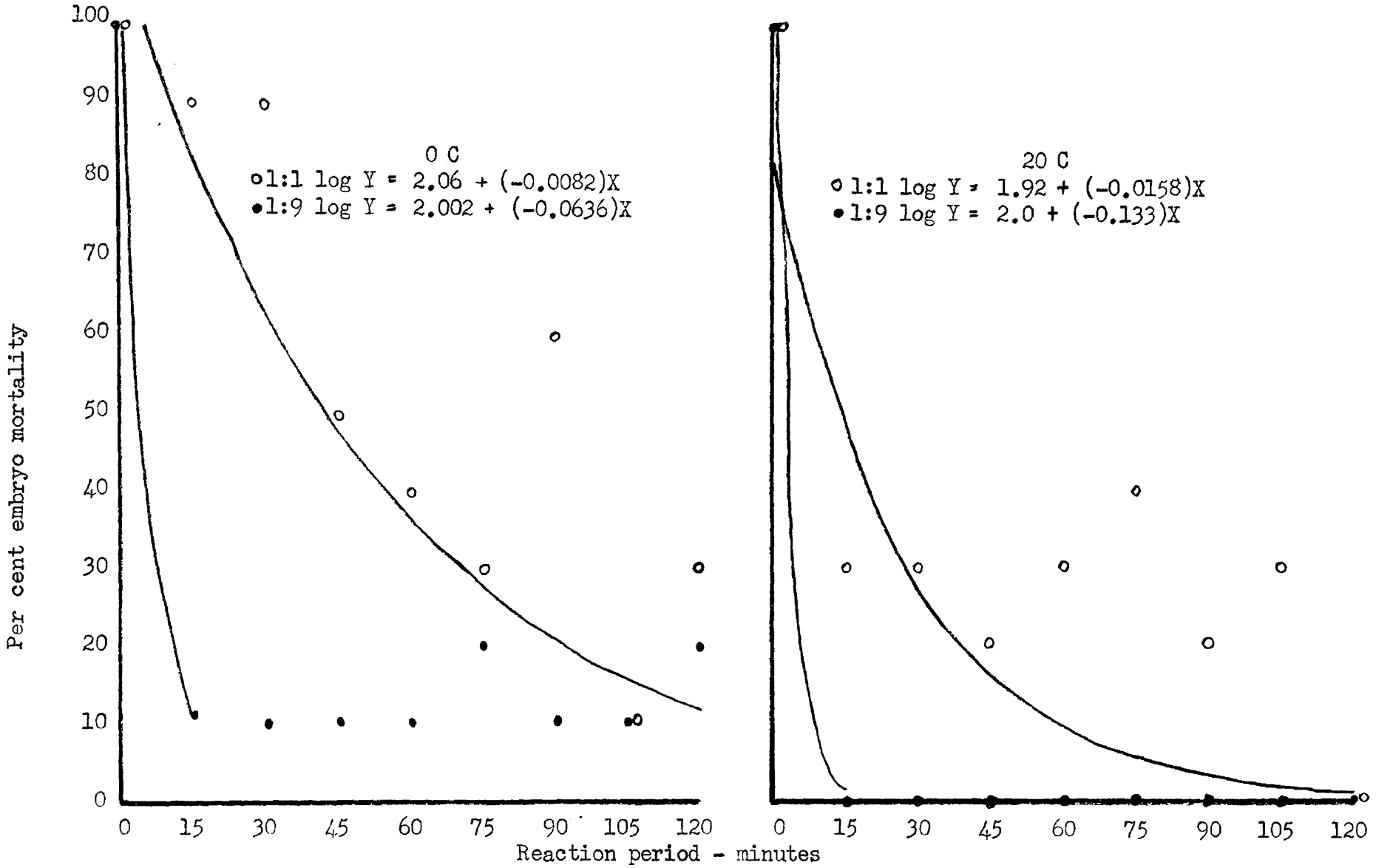

Figure 5. Effect of sodium hydroxide (1%), pH 13.0, on Newcastle disease virus, l.d.$_{50}$ $10^{8.8}$, in proportions of virus:sodium hydroxide of 1:1 and 1:9 at 0 C and 20 C.

minutes after contact of the virus and sodium hydroxide, the mortality was 30 per cent. At 45 minutes the mortality was 20 per cent, 60 minutes 30 per cent, 75 minutes 40 per cent, 90 minutes 20 per cent and no mortality at 120 minutes. The general pattern indicates an initial rapid inactivation during the first 45 minutes followed by a 60 minute interval in which there was a fluctuation in viral activity and finally a complete inactivation of the virus at 120 minutes. With the exception of the 60 to 105 minute interval, the rate of inactivation fits reasonably well a logarithmic curve as shown by the equation $\log Y = 1.92 + (-0.0158)X$. With the 1:9 mixture, the virus was completely inactivated 15 minutes after contact with sodium hydroxide. The equation $\log Y = 2.0 + (-0.133)X$ fits these data.

From these data for 0 C and 20 C exposures, it is apparent that the rate of inactivation was greater in the 1:9 mixtures than in the 1:1 mixtures and both were subject to thermal influence with a more rapid rate of inactivation at the higher temperature.

At 37 C only the 1:1 mixture of virus and sodium hydroxide was employed as the previous results with the 1:9 mixture at 20 C indicated that the rate of inactivation would be too rapid for practical evaluation. The mortality 15 minutes after contact was 20 per cent followed by survival of all embryos at the 30 minute period. At 45 minutes 20 per cent of the embryos were killed, ten per cent at 60 minutes and no mortality at the 75 to 120 minute intervals. Figure 6. Using the data through the 75 minute period with the exception of no mortality at 30 minutes, which evidently is not valid by reason of subsequent mortality at the two succeeding periods, the curve $\log Y = 1.94 + (-0.021)X$ is obtained. This

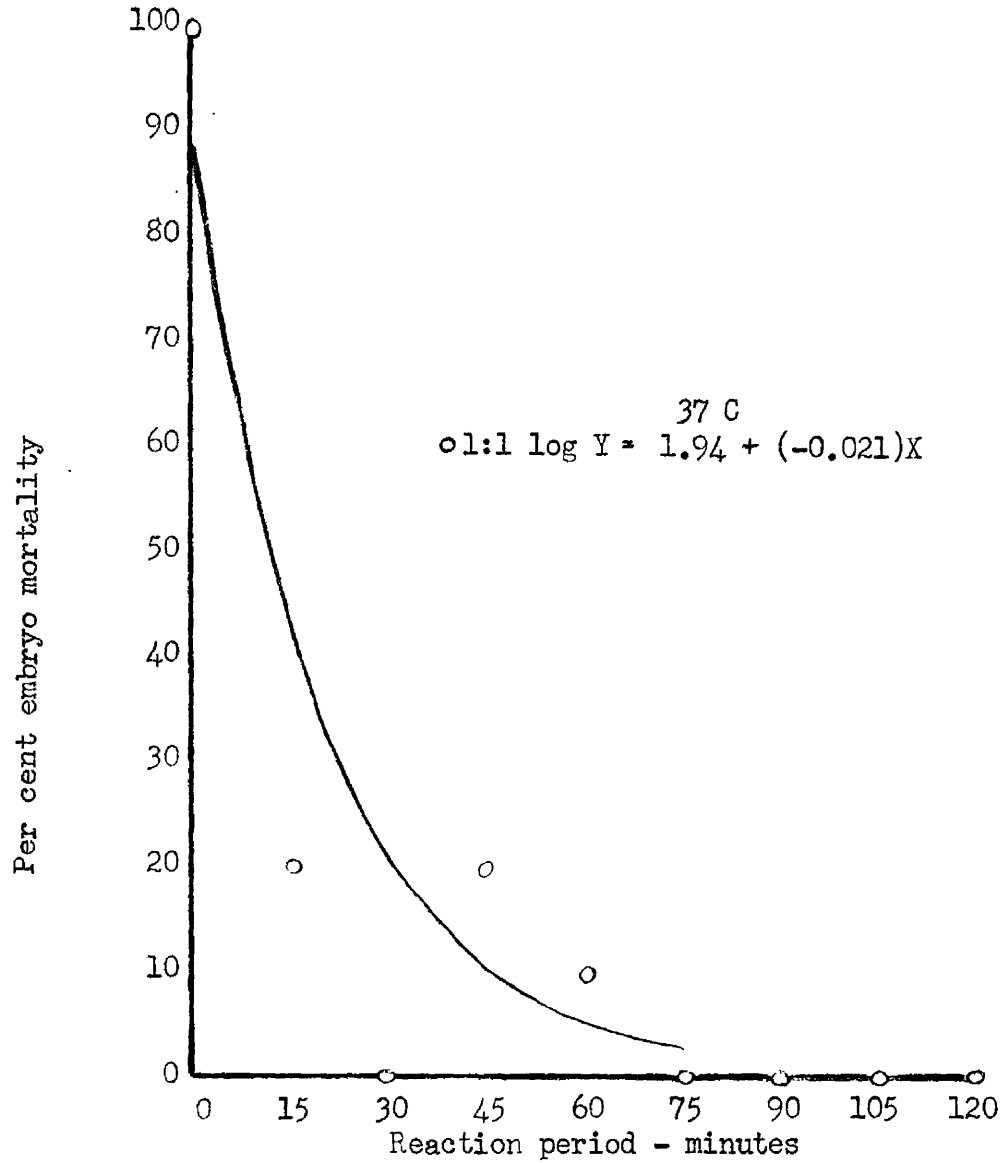

Figure 6. Effect of sodium hydroxide (1%), pH 13.0, on Newcastle disease virus, l.d.$_{50}$ $10^{8.8}$, in proportions of virus:sodium hydroxide of 1:1 at 37 C.

curve does not fit the data too well, especially at the time of contact, but does serve to indicate a logarithmic phase of inactivation of the virus.

From these data for sodium hydroxide, it is evident that when the virus and agent were mixed in equal parts, the rate of reaction was logarithmic and there was a direct thermal influence. At 0 C complete inactivation had not occurred at 120 minutes. At 20 C inactivation occurred at 120 minutes and at 37 C this was attained at 75 minutes. With the 1:9 mixtures the rate of inactivation was extremely rapid within 15 minutes after contact of the virus and agent at 0 C and 20 C. In the former, complete inactivation did not occur at 120 minutes whereas in the latter complete inactivation occurred within 15 minutes.

NDV mixed with sodium hypochlorite, 440 p.p.m. available chlorine, in equal parts at 0 C was not inactivated during 120 minutes exposure as shown in Figure 7 with the equation $\log Y = 2.0 + 0.0X$. When mixed in proportions of 1:9 the virus was quickly inactivated. Fifteen minutes after contact the mortality was ten per cent followed by no mortality at 30 minutes. At 45 and 60 minutes the mortality was ten per cent, no mortality at 75 through 105 minutes, and ten per cent mortality at 120 minutes. Using the data through the 30 minute period, assuming that the minor fluctuations after this period might possibly be due to technic, the equation $\log Y = 1.99 + (-0.066)X$ is obtained which fits the data well and indicates a logarithmic rate of inactivation.

At 20 C, inactivation of the virus was not detected in the mixture of equal parts exposed for 120 minutes. Figure 7. The equation is $\log Y = 2.0 + 0.0X$. The rate of inactivation in the 1:9 mixture was similar

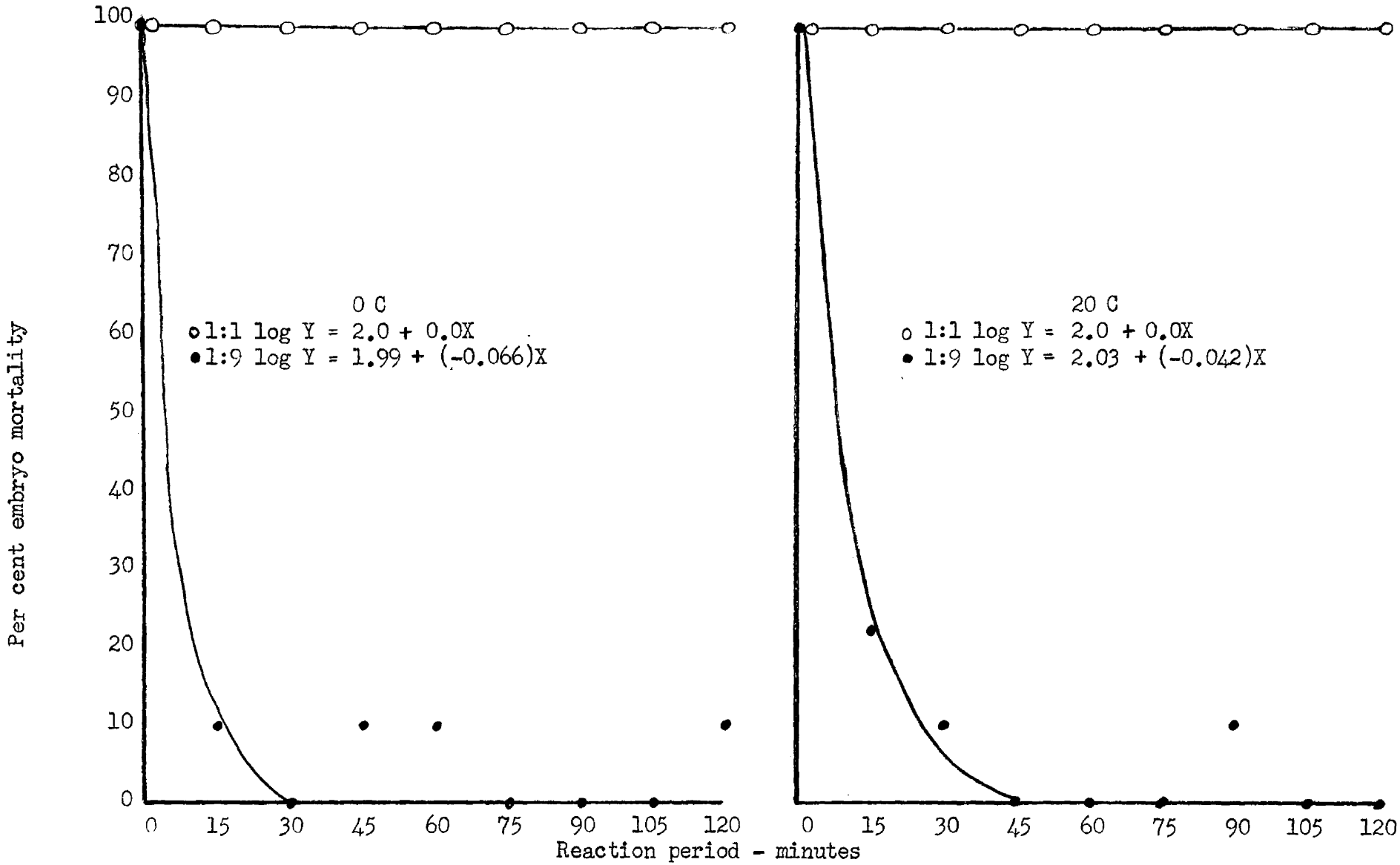

Figure 7. Effect of sodium hypochlorite (440 p.p.m. available chlorine), pH 10.9, on Newcastle disease virus, $l.d._{50}$ $10^{8.8}$, in proportions of virus:sodium hypochlorite of 1:1 and 1:9 at 0 C and 20 C.

to that in the same mixture at 0 C. Fifteen minutes after contact, 22.2 per cent of the embryos (two of nine embryos) were killed by the virus. At 30 minutes ten per cent of the embryos died but at 45 through 75 minutes none of the embryos were killed. Ten per cent died at 90 minutes and none at 105 and 120 minutes. Using the data through the 45 minute reaction period, the mortality at 90 minutes not being considered significant, the equation $\log Y = 2.03 + (-0.042)X$ fits the data well and indicates the logarithmic regression of activity of the virus. It is obvious in the 0 C and 20 C tests that the rate of inactivation with the 1:9 mixtures was much greater than with the 1:1 mixtures.

At 37 C the effect of thermal influence was quite marked. Figure 8. Fifteen minutes after contact of the virus and the agent in the 1:9 mixture, 11.1 per cent of the embryos (one of nine embryos) died and none were killed at the 30 and 45 minute periods. Sixty minutes after contact, 11.1 per cent of the embryos died. No mortality occurred at the 75 minute interval but 20 and ten per cent mortality was produced at the 90 and 105 minute periods, respectively. Using the data through the 30 minute exposure as an indication of the period of inactivation, the curve $\log Y = 2.014 + (-0.066)X$ fits these data well. It is assumed that the variability in mortality during the latter stages of exposure was due to nonspecific causes. With the mixture of equal parts of virus and agent, all embryos died during the first 30 minutes. Ninety per cent were killed at the 45 minute period, 55.5 per cent (five of nine embryos) at 60 minutes, 40 per cent at 75 minutes, 20 per cent at 90 minutes, and 30 and ten per cent, respectively, at the 105 and 120 minute periods. The equation $\log Y = 2.04 + (-0.0103)X$ fits the data well throughout the entire period of inactivation.

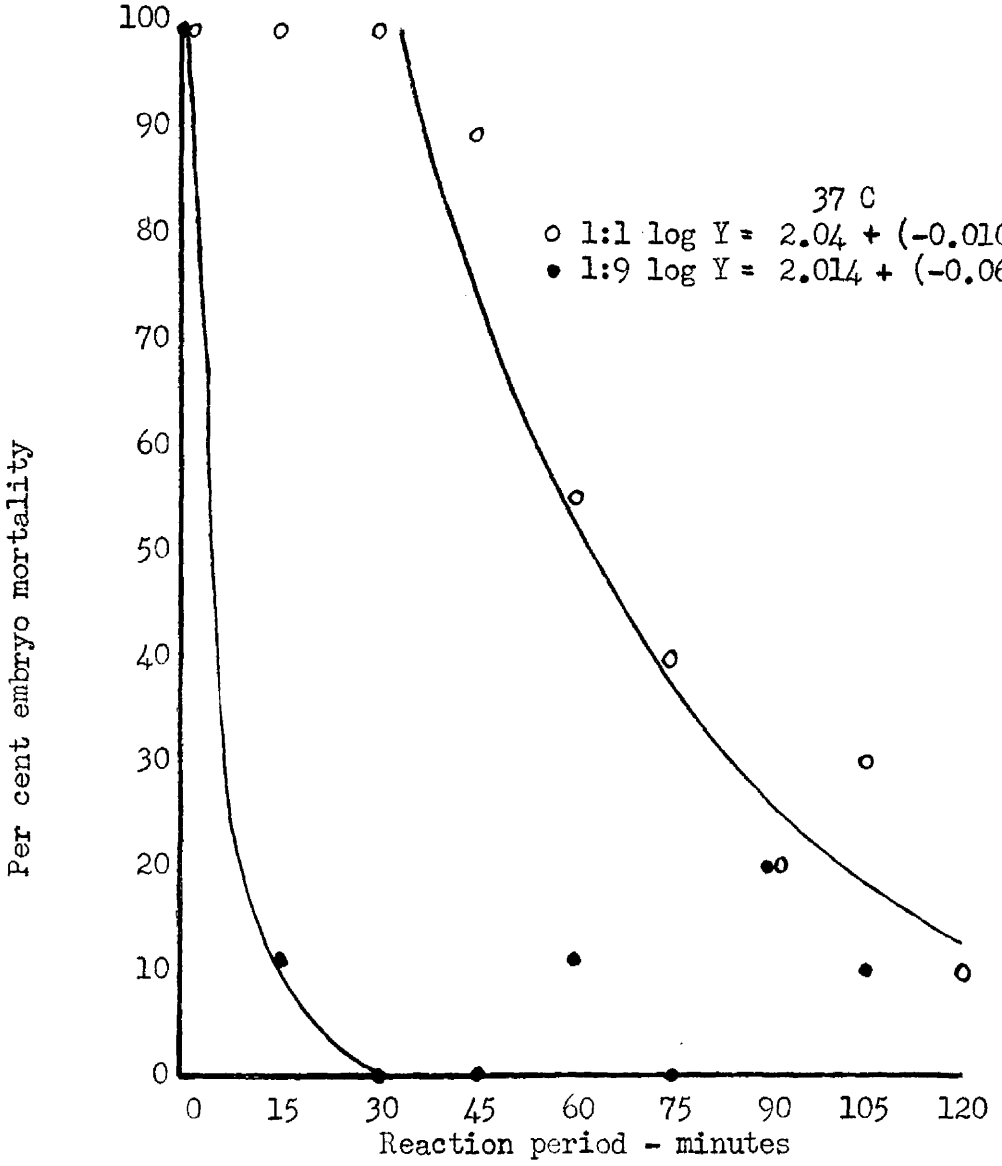

Figure 8. Effect of sodium hypochlorite (440 p.p.m. available chlorine), pH 10.9, on Newcastle disease virus, $l.d._{50}$ $10^{8.8}$, in proportions of virus:sodium hypochlorite of 1:1 and 1:9 at 37 C.

These data for sodium hypochlorite show that in the 1:9 mixtures at 0 C, 20 C and 37 C, the rate of inactivation was similar and not markedly influenced by the different temperatures of exposure. With the mixture of equal parts of NDV and sodium hypochlorite, thermal influence was first detected with the tests at 37 C as shown by the logarithmic regression of embryo mortality at the 30 minute and subsequent exposure periods.

NDV with Roccal, 0.01 per cent, in proportions of 1:1 and 1:9 at 0 C and 20 C was capable of killing all embryos during the 90 minute exposure period. Figure 9. At 37 C, the mixture of equal parts killed all embryos for 45 minutes but at the 60 minute period 90 per cent of the embryos died. At 75 minutes the mortality was 100 per cent and at 90 minutes 80 per cent. With these limited data which show slight inactivation of the virus, the equation $\log Y = 1.99 + (-0.00214)X$ fits the data reasonably well. With the 1:9 mixture inactivation was most abrupt. Fifteen minutes after contact the virus was completely inactivated as evidenced by survival of all embryos. At 30 minutes none of the embryos died. Ten per cent mortality was observed at the 45, 75 and 90 minute periods but no mortality at the 60 minute period. The equation $\log Y = 2.0 + (-0.1333)X$ illustrates the inactivation rate.

With mercuric chloride, 0.01 per cent, and tincture of metaphen, 0.01 per cent, in proportions of virus:chemical agent of 1:1 and 1:9 at 0 C, 20 C and 37 C the embryo mortality was 100 per cent in all tests for 120 minutes. The equation $\log Y = 2.0 + 0.0X$ fits these data as shown in Figures 10 and 11, respectively.

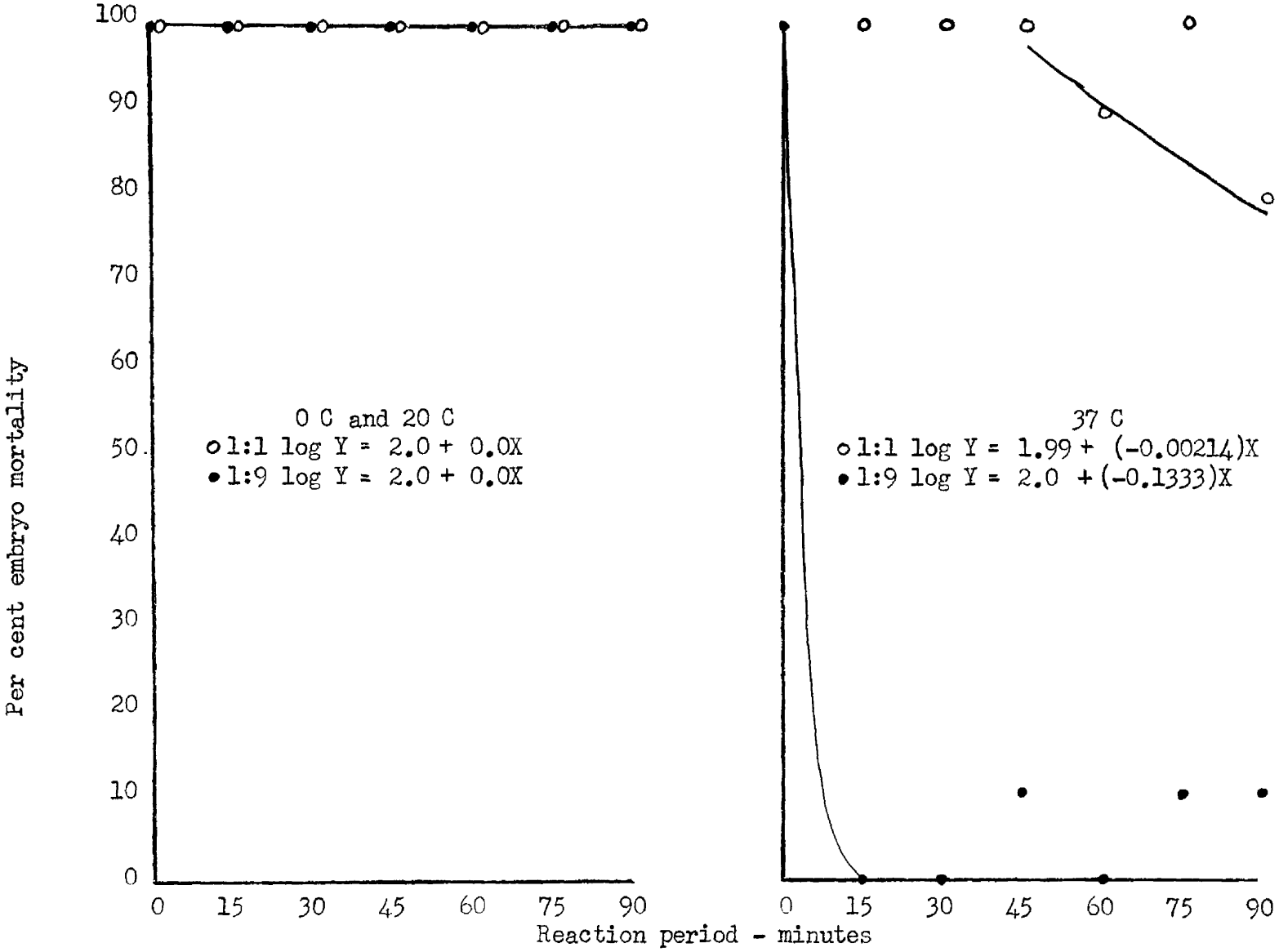

Figure 9. Effect of Roccal (0.01%), pH 6.1, on Newcastle disease virus, l.d.$_{50}$ $10^{8.8}$, in proportions of virus:Roccal of 1:1 and 1:9 at 0 C, 20 C and 37 C.

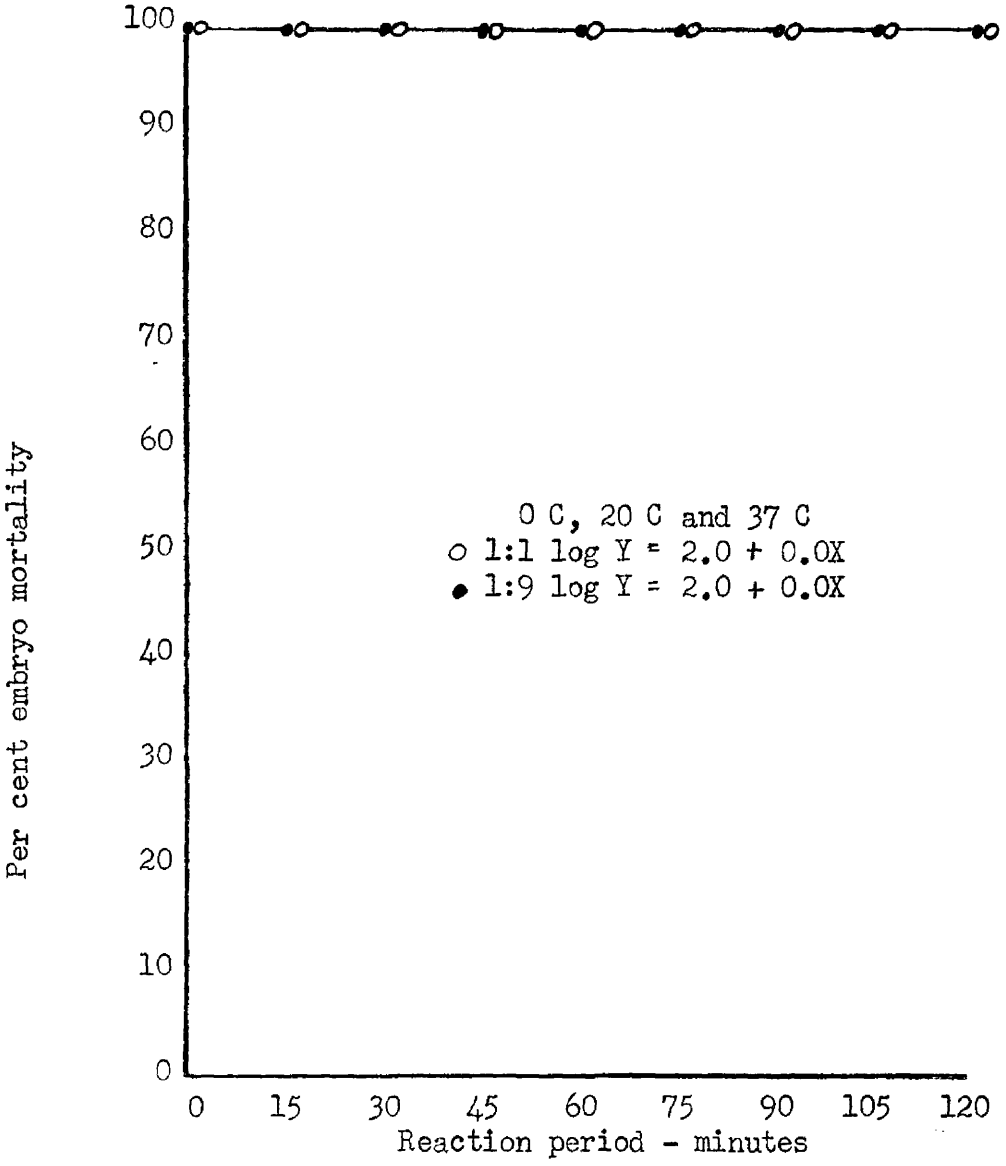

Figure 10. Effect of mercuric chloride (0.01%), pH 3.5, on Newcastle disease virus, $l.d._{50}$ $10^{8.8}$, in proportions of virus:mercuric chloride of 1:1 and 1:9 at 0 C, 20 C and 37 C.

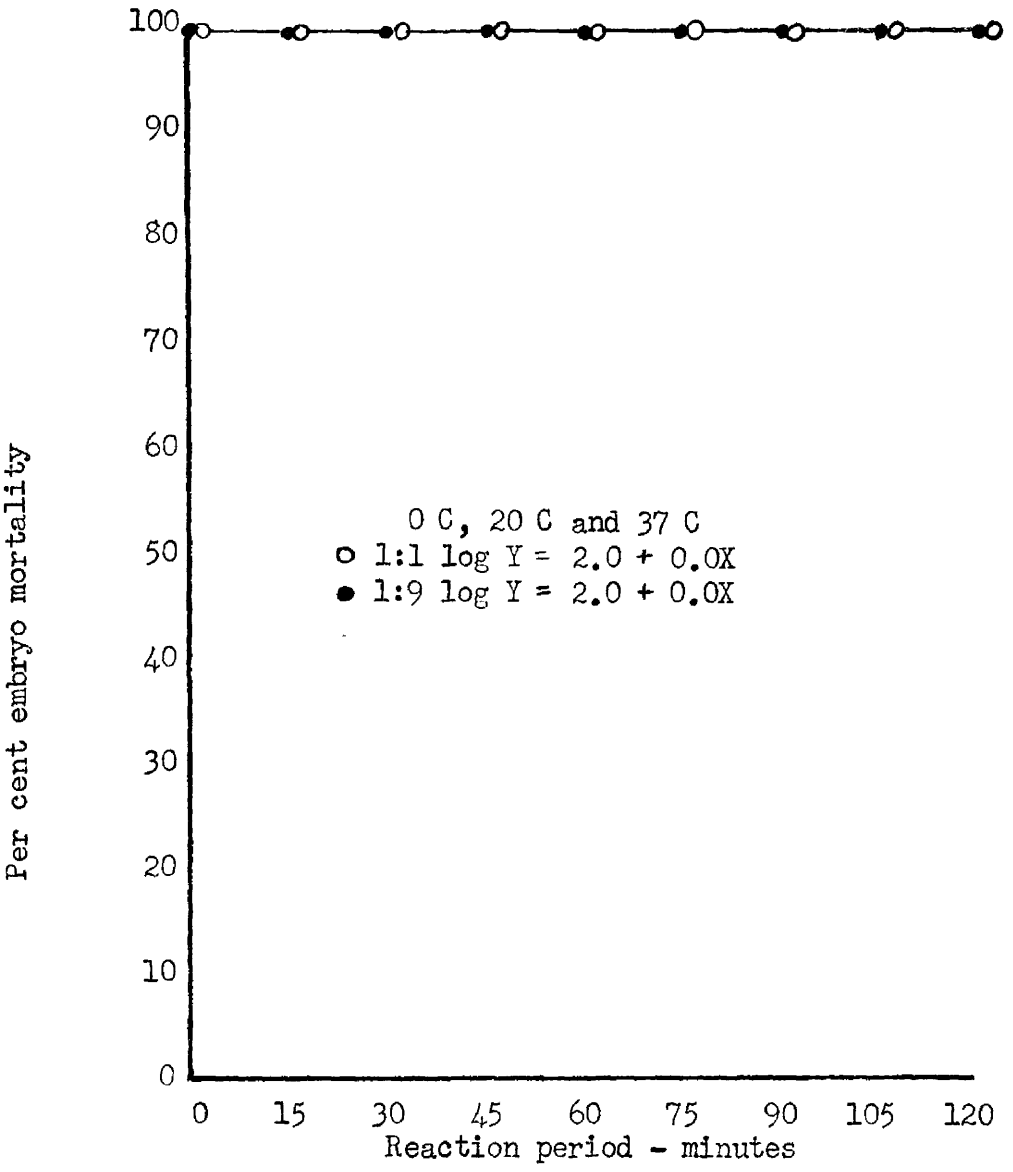

Figure 11. Effect of tincture of metaphen (0.01%), pH 8.4, on Newcastle disease virus, $l.d._{50}$ $10^{8.8}$, in proportions of virus:tincture of metaphen of 1:1 and 1:9 at 0 C, 20 C and 37 C.

Effect of Chemical Agents on Different Concentrations of Newcastle Disease Virus

For this part of the study, the virus was titrated by using serial ten-fold aqueous dilutions with ten embryos per dilution. The results of the titration are shown in Figure 12. In the 10^{-8} dilution the embryo mortality was 100 per cent, 10^{-9} 50 per cent, and 10^{-10} 20 per cent. The $l.d._{50}$ of the virus was $10^{9.2}$. The equation for decrease of viral activity with increasing dilution is log Y = 2.0166 + 0.3495X. This equation and all others were compiled by assigning 0.0X to the highest dilution (lowest concentration) of the virus in which mortality was 100 per cent. The successive dilutions (concentrations) were assigned the values -1.0X, -2.0X, et cetera, through the lowest dilution (concentration) in which no mortality occurred. The above equation fits extremely well the observed data for titration of the virus and illustrates a logarithmic regression of embryo mortality based on serial ten-fold dilutions of the virus.

The results of the effect of the several chemical agents on different concentrations of NDV in proportions of virus:chemical agent of 1:9 for 15 minutes at 20 C are presented in Table III. One part of each virus sample was mixed with nine parts of chemical agent and a 10^{-2} dilution of this mixture was made prior to injection of eggs. This resulted in a 10^{-3} dilution of the virus sample initially mixed with the chemical agent. For quantitative assay of the number of lethal doses of virus completely inactivated by the chemical agent, the end-point of embryo mortality would have to be considered as 10^{-3} less than the concentration of the virus initially mixed with the chemical agent. As previously determined by

TABLE III

PER CENT EMBRYO MORTALITY FOLLOWING INOCULATION WITH MIXTURES OF DIFFERENT CONCENTRATIONS OF NEWCASTLE DISEASE VIRUS, $l.d._{50}$ $10^{9.2}$, AND CERTAIN CHEMICAL AGENTS. REACTION PERIOD 15 MINUTES AT 20 C. PROPORTIONS OF VIRUS: CHEMICAL AGENT 1:9.

Chemical Agent	Log concentration of virus						
	9.2	8.2	7.2	6.2	5.2	4.2	3.2
Phenol, 1%, pH 6.4	100	100	100	100	20	0	0
Ethyl alcohol, 40%, pH 6.9	100	20	20	10	0	0	0
Sodium hydroxide, 1%, pH 13.0	10	0	0	0	0	0	0
Sodium hypochlorite, 440 p.p.m., available chlorine, pH 10.9	0	0	0	0	0	10	0
Roccal, 0.01%, pH 6.1	10	0	0	0	0	0	0
Mercuric chloride, 0.01%, ph 3.5	100	100	100	100	0	0	0
Tincture of metaphen, 0.01%, pH 8.4	100	100	100	100	100	100	30

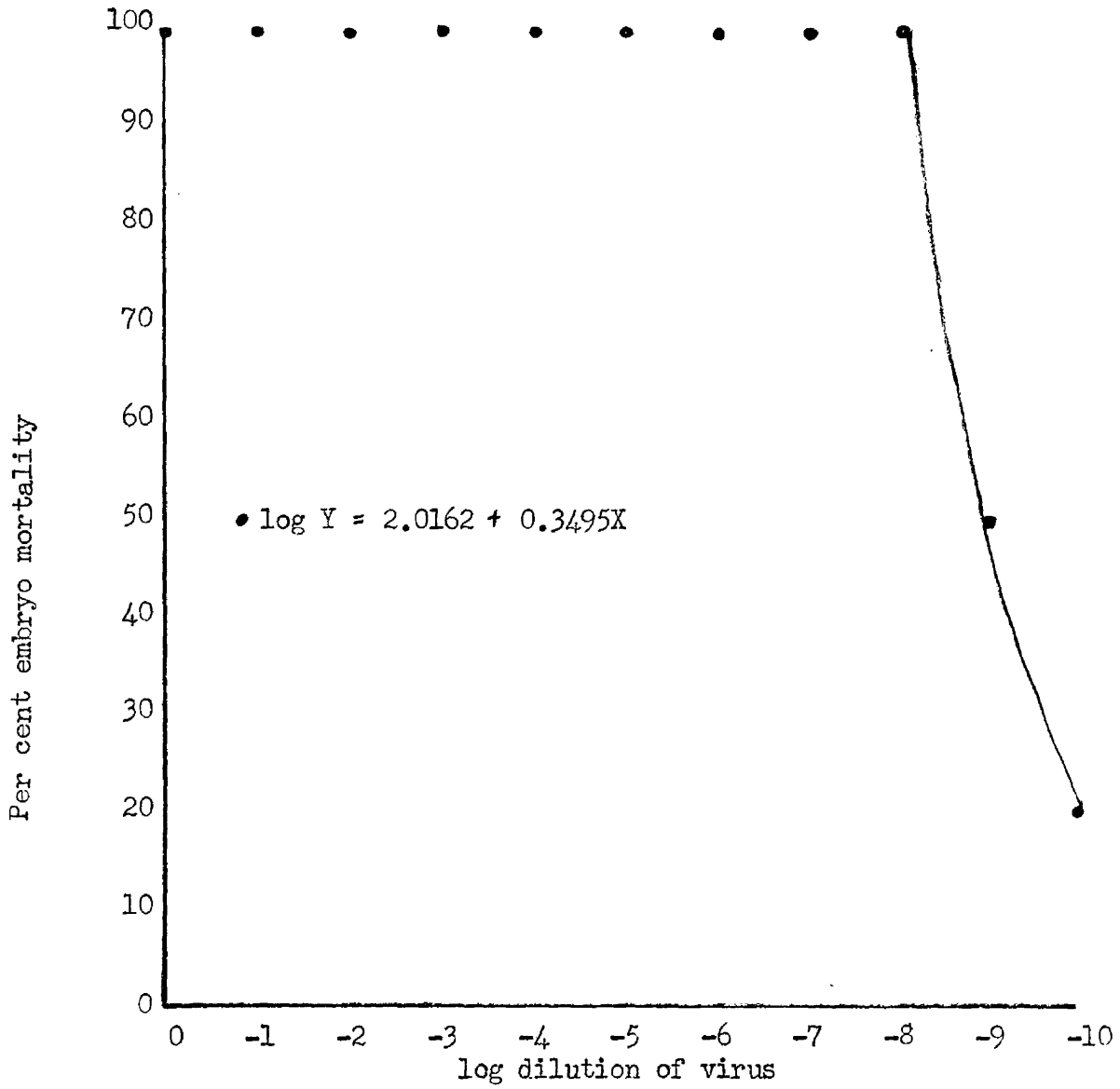

Figure 12. Titration of Newcastle disease virus, l.d.$_{50}$ $10^{9.2}$, in ten-day embryonating chicken eggs. Inoculum 0.1 ml per egg.

titration, 100 per cent embryo mortality would be obtained with at least $10^{1.2}$ lethal doses of the virus which would be contained in the 10^{-8} dilution of the l.d.$_{50}$ $10^{9.2}$ virus. Figure 12. In all tests, except that with tincture of metaphen, this dilution factor did not have to be considered in computing the rates of inactivation of the virus as the virus in concentrations greater than $10^{1.2}$ had been completely inactivated by the chemical agent.

When the undiluted virus, $10^{9.2}$ doses, was mixed with ethyl alcohol, 40 per cent, the mortality was 100 per cent. Figure 13. When $10^{8.2}$ and $10^{7.2}$ doses were employed the mortality was 20 per cent. Ten per cent of the embryos were killed when $10^{6.4}$ doses were used but none were killed when $10^{5.2}$ to $10^{3.2}$ doses were used. The latter range indicates complete inactivation of the virus in the concentrations employed. The equation $\log Y = 1.98 + 0.43X$ fits these data of observed mortality rates and indicates a logarithmic rate of inactivation of the virus. The chemical could be considered as having completely inactivated as many as 158,500 doses of the virus.

With phenol, one per cent, 100 per cent embryo mortality was obtained with virus concentrations ranging from $10^{9.2}$ through $10^{6.2}$. Figure 13. Ten per cent mortality occurred with $10^{5.2}$ doses of virus and no mortality with $10^{4.2}$ and $10^{3.2}$ doses. The equation $\log Y = 2.1 + 1.0X$ fits well the observed data. Complete inactivation of 15,850 doses was produced by phenol.

With mercuric chloride, 0.01 per cent, all embryos died in virus concentrations ranging from $10^{9.2}$ through $10^{6.2}$ and no mortality was produced with $10^{5.2}$ or fewer doses. Figure 14. These data fit well the equation

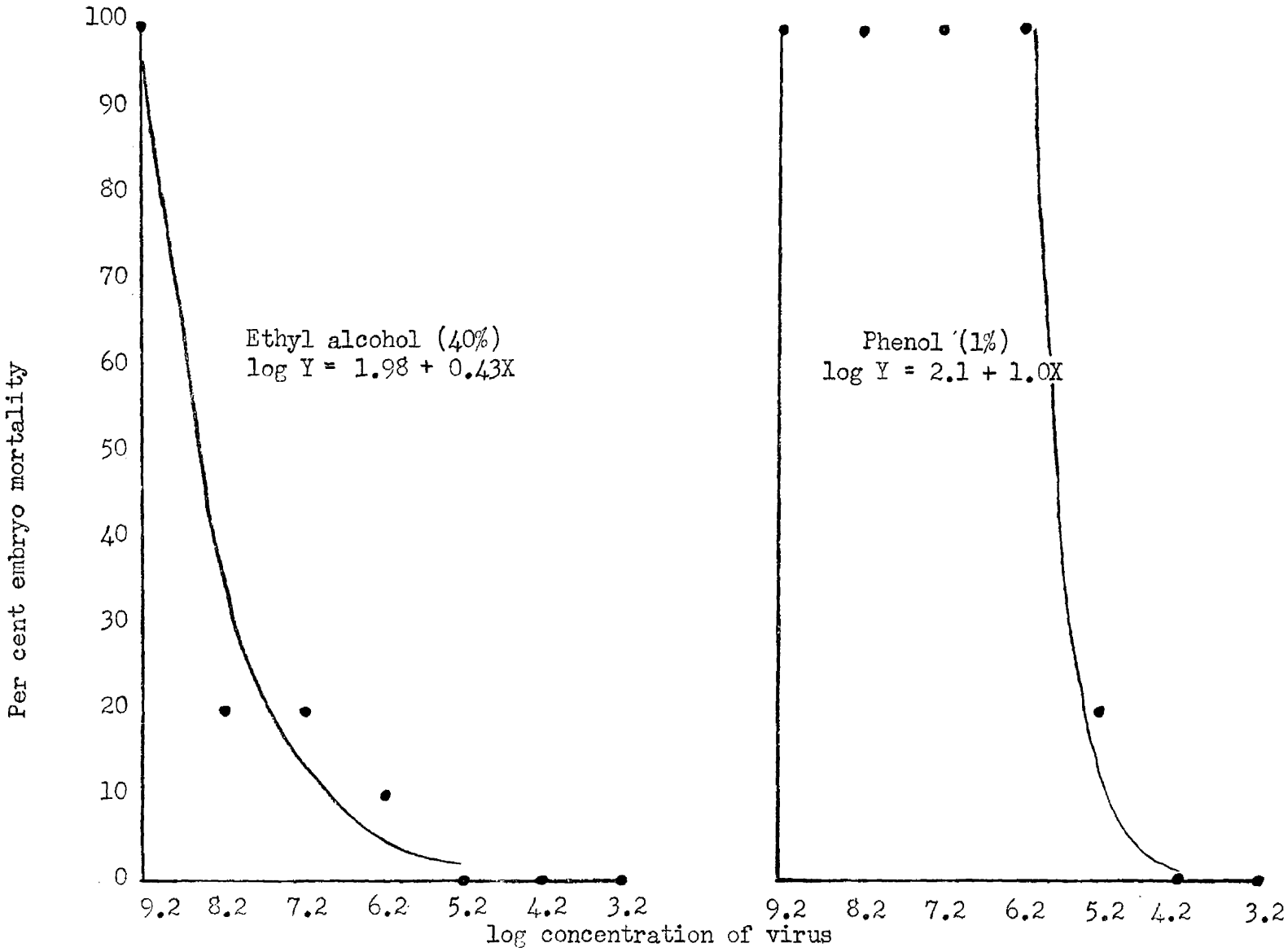

Figure 13. Effect of certain chemical agents on different concentrations of Newcastle disease virus, $l.d._{50}$ $10^{9.2}$, at 20 C for 15 minutes in proportions of virus:chemical agent of 1:9.

log Y = 2.0 + 2.0X and indicate that mercuric chloride completely inactivated as many as 158,500 doses of virus.

Sodium hydroxide, one per cent, and Roccal, 0.01 per cent, exerted a rapid effect on NDV and at the same rate. Figures 14 and 15. When $10^{9.2}$ doses of virus were mixed with these chemical agents, there was ten per cent mortality of the embryos followed by no mortality with $10^{8.2}$ or fewer doses. These data, while insufficient for analysis of the rate of inactivation, suggest a logarithmic regression as there was a 90 per cent reduction of embryo mortality from the time of contact of the virus and chemical agent to the sampling period. These results indicate that these chemical agents inactivated as many as 158,500,000 doses of virus.

Sodium hypochlorite, 440 p.p.m. available chlorine, completely inactivated the virus in all concentrations of virus employed from $10^{9.2}$ through $10^{3.2}$ with the exception of the $10^{4.2}$ concentration. This latter concentration in which ten per cent mortality occurred is not considered significant in evaluating the results. These data indicate that sodium hypochlorite, 440 p.p.m. available chlorine, completely inactivated 1,585,000,000 doses of the virus from the time of contact to the sampling period. The rate of inactivation was probably a logarithmic regression.

Tincture of metaphen, 0.01 per cent, did not inactivate the virus in concentration of $10^{9.2}$ through $10^{4.2}$ as evidenced by 100 per cent embryo mortality in these dilutions. With $10^{3.2}$ doses of virus the mortality was 30 per cent. Using the dilution factor of 10^{-3} as previously described for evaluating rates of inactivation, this would indicate that only $10^{0.2}$ doses of virus were injected into the eggs. Comparison of the 30 per cent mortality obtained with this concentration of NDV and tincture of metaphen

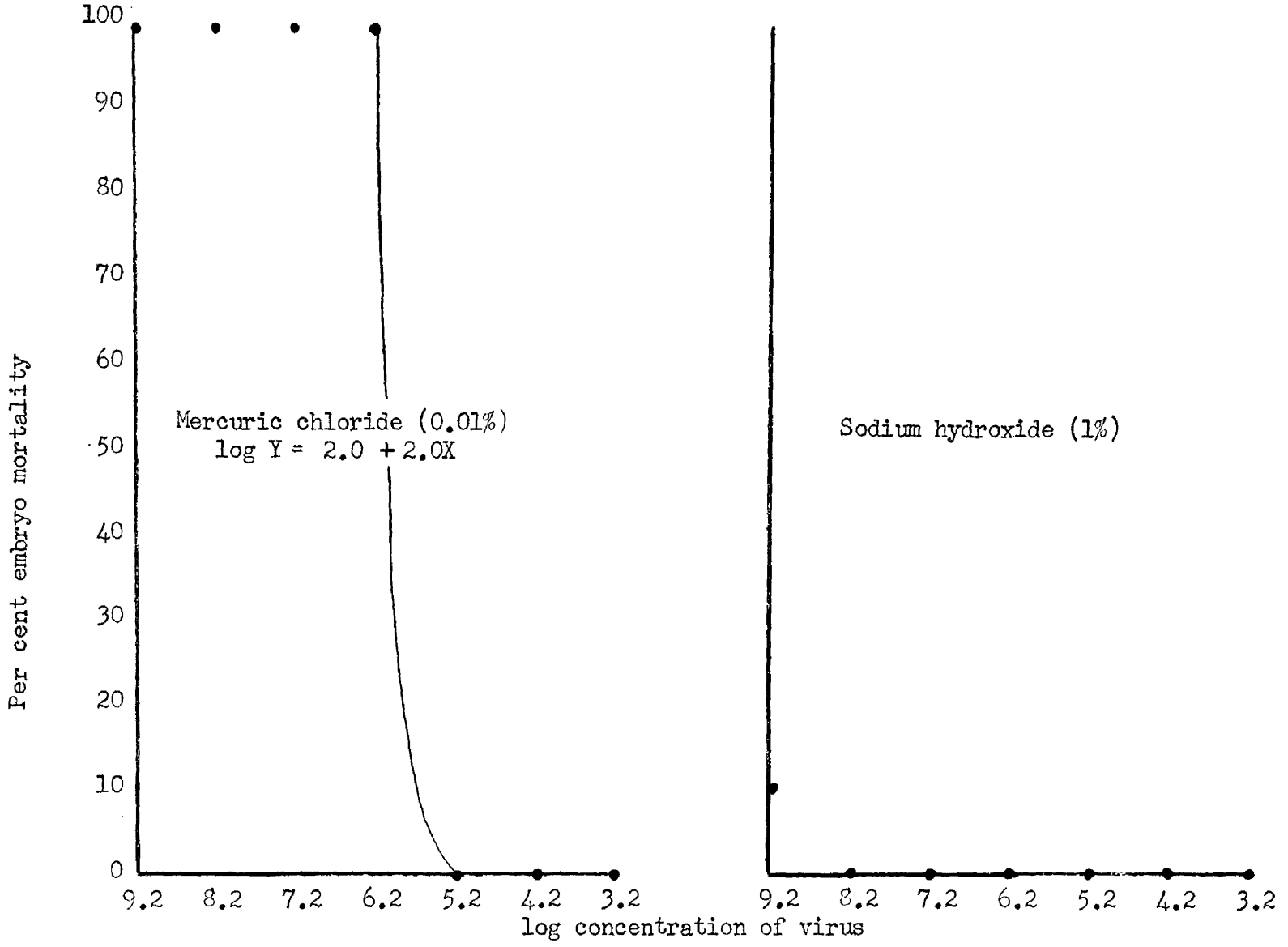

Figure 14. Effect of certain chemical agents on different concentrations of Newcastle disease virus, $l.d._{50}$ $10^{9.2}$, at 20 C for 15 minutes in proportions of virus: chemical agent of 1:9.

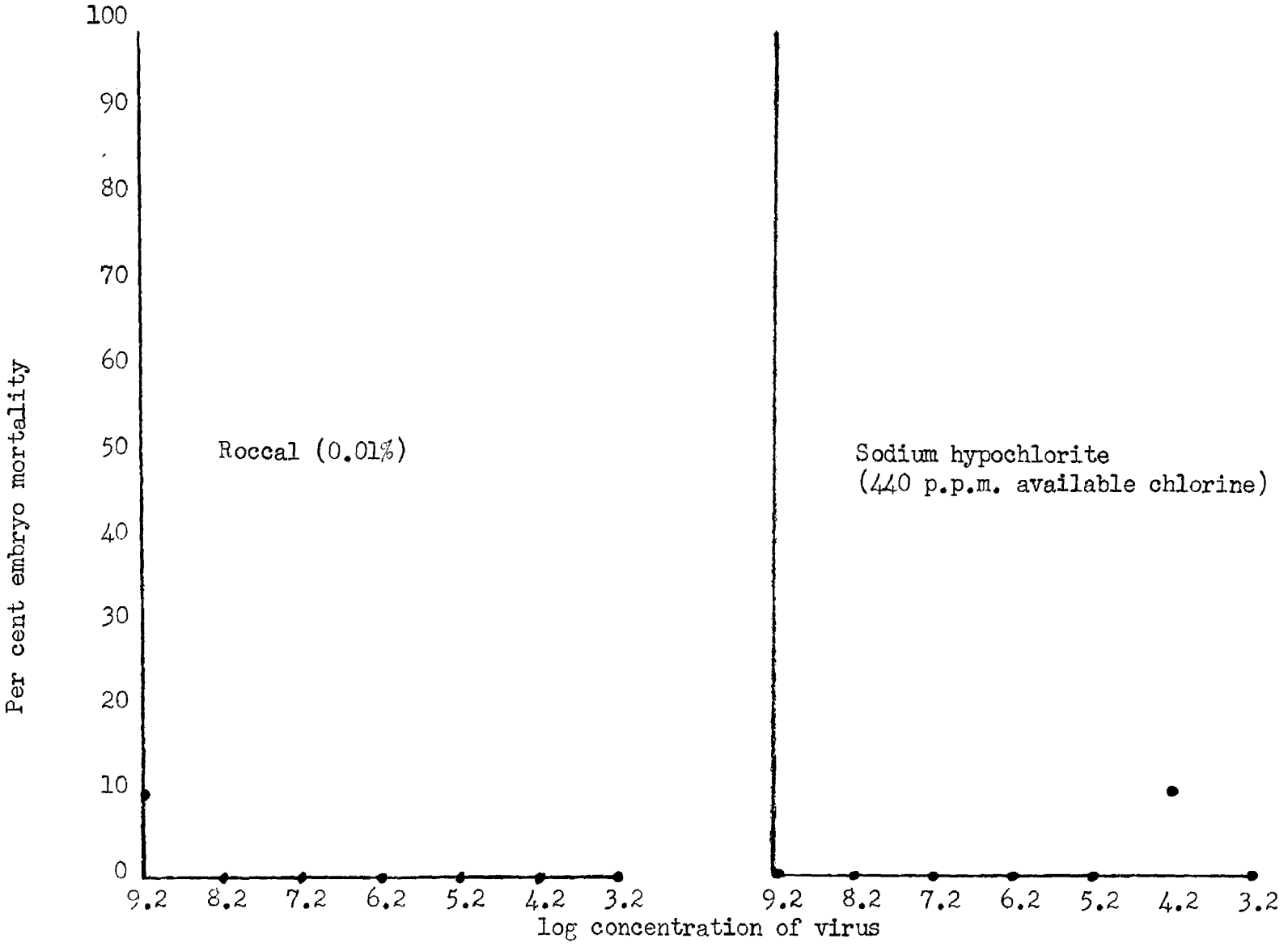

Figure 15. Effect of certain chemical agents on different concentrations of Newcastle disease virus, l.d.$_{50}$ $10^{9.2}$, at 20 C for 15 minutes in proportions of virus: chemical agent of 1:9.

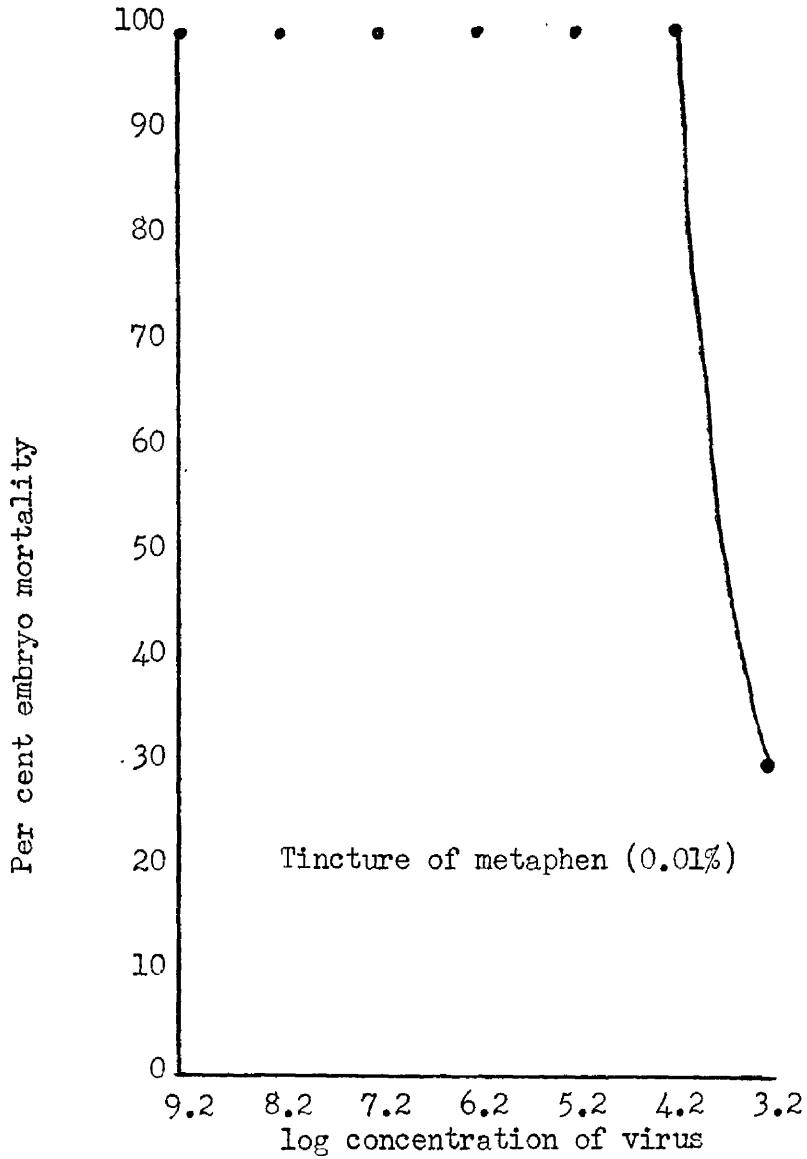

Figure 16. Effect of certain chemical agents on different concentrations of Newcastle disease virus, l.d.$_{50}$ $10^{9.2}$, at 20 C for 15 minutes in proportions of virus: chemical agent of 1:9.

with the 50 per cent mortality obtained in the virus titration shows only a 20 per cent differential in the mortality rates. This is but a slight effect and tincture of metaphen would necessarily be considered as completely inactivating less than two doses of virus.

V

DISCUSSION

The effect of some of the chemical agents employed in these studies could not be detected as all embryos were killed at each period following inoculation with the virus-chemical agent mixtures. The agent in the concentration employed was probably unable to influence the virus to the extent that it was not capable of killing all embryos. In those tests in which embryo mortality was less than 100 per cent, the rate of inactivation of the virus could be measured quantitatively and expressed as the coefficient of regression from the equation $\log Y = a + bX$. One exception occurred with phenol, one per cent, 1:9 mixture, 0 C. The rate of inactivation in this test was best expressed as a linear function from the equation $Y = a + bX$.

The undiluted virus-infected allantoic fluid preparation used in these studies contained 0.54 mg nitrogen per ml as determined by the macro Kjeldahl method. The allantoic cavity serves as a receptacle for nitrogenous waste products of the chicken embryo. The largest single fraction of this nitrogen is due to the presence of uric acid, the chief excretory product of birds. According to Romanoff (1952), uric acid nitrogen may constitute as much as 74 per cent by the twelfth day of incubation. The virus preparation used in these studies was collected from 12-day-old embryonating chicken eggs. While the factor of 6.25 cannot be used with accuracy for estimating the protein content of the virus preparation used, it would indicate about 3.375 mg of protein per ml. The preparation employed in these studies was far from being a "pure"

preparation of NDV but did represent the type of specimen used for comparative evaluation of the effect of chemical agents on the virus.

The errors inherent in this method of evaluating the effect of chemical agents make too close mathematical analyses unjustified but certain trends of inactivation of the virus are evident from these analyses. In all tests in which the virucidal properties of the agents could be detected, the initial stage of inactivation for the 1:9 mixtures occurred between the time of contact of the virus and the agent and the first sampling period. This period was ten minutes for phenol, one per cent, at 20 C, ethyl alcohol, 40 per cent, at 20 C and 37 C; and 15 minutes for sodium hydroxide, one per cent, at 0 C and 20 C, sodium hypochlorite, 440 p.p.m. available chlorine, at 0 C, 20 C and 37 C, and Roccal, 0.01 per cent, at 37 C. With phenol, one per cent at 37 C the first sampling period was at 20 minutes. With the 1:1 mixtures, the initial stage of inactivation could first be detected between the 30 and 40 minute intervals after contact with phenol, one per cent, at 0 C; 40 to 50 minutes for ethyl alcohol, 40 per cent, at 37 C; 15 minutes for sodium hydroxide, one per cent, at 0 C, 20 C and 37 C; 30 to 45 minutes for sodium hypochlorite, 440 p.p.m. available chlorine, at 37 C, and 45 to 60 minutes for Roccal, 0.01 per cent, at 37 C. These data show that the initial detectable stage of inactivation of the virus occurred at an earlier period in the 1:9 mixtures than in the 1:1 mixtures with the exception of sodium hydroxide, one per cent, in which the initial stage occurred during the same period in both mixtures.

For comparison of the rates of inactivation as influenced by proportions of virus and chemical agent in the 1:1 and 1:9 mixtures, only those

tests may be utilized in which inactivation occurred in both mixtures at the same temperature. The amount of agent in the 1:9 mixtures was five times greater than in the 1:1 mixtures. With phenol, one per cent, at 20 C, the coefficient of regression for the 1:1 mixture was -0.1 or approximately two times that of -0.0478 for the 1:9 mixture. The coefficient of regression for ethyl alcohol, 40 per cent, at 37 C was -0.043 for the 1:9 mixture or approximately two times that of -0.02 for the 1:1 mixture. With sodium hydroxide, one per cent, the coefficients of regression for the 1:9 and 1:1 mixtures, respectively, were -0.0636 and -0.0082 at 0 C or approximately 7.7 times greater for the 1:9 mixture. At 20 C, the coefficients of regression were -0.133 for the 1:9 mixture and -0.0158 for the 1:1 mixture or approximately 8.4 times greater for the 1:9 mixture. The ratio of the coefficients of regression for sodium hypochlorite, 440 p.p.m. available chlorine, at 37 C were -0.066 for the 1:9 mixture and -0.0103 for the 1:1 mixture or approximately 6.4 times greater for the 1:9 mixture. The data for Roccal, 0.01 per cent at 37 C for the 1:1 mixture are not considered of significant value for comparison with that for the 1:9 mixture. The above data indicate that the rate of inactivation for the 1:9 mixtures was greater than for the 1:1 mixtures with the exception of phenol, one per cent, where the reverse occurred.

That increased temperatures augment the action of alcohol is evidenced in the case of ethyl alcohol, 40 per cent, in which the coefficient of regression of the 1:9 mixture at 20 C was -0.003 and at 37 C was -0.043, or 14.3 times greater with an increase of 17 C.

That sodium hydroxide solutions at lower temperatures are virucidally efficacious is shown with sodium hydroxide, one per cent, in which the coefficient of regression for the 1:1 mixture was -0.0082 at 0 C and -0.0158 at 20 C or approximately 1.93 times greater with an increase of 20 C. With the 1:9 mixture the coefficient of regression at 0 C was -0.0636 and at 20 C was -0.133 or approximately 2.1 times greater with an increase of 20 C. Comparison of the coefficient of regression for the 1:1 mixture at 20 C, -0.0158, and at 37 C, -0.021, shows an increase of about 1.3 with an increase of 17 C.

Sodium hypochlorite, 440 p.p.m. available chlorine, was also efficacious at low temperatures as shown by the coefficients of regression at 0 C, -0.066, 20 C, -0.042, and 37 C, -0.066, which were approximately the same with the 1:9 mixtures.

The data presented as to the effect of certain chemical agents on undiluted virus-infected allantoic fluid indicated a logarithmic rate of inactivation. Investigations of the effect of the chemical agents on different concentrations of the virus substantiate these findings. In all instances in which the observed data were of sufficient magnitude for analysis, the decrease in viral activity was logarithmic.

The organic matter in the virus preparation employed could have interferred with the virucidal activity of the chemical agents, particularly with sodium hypochlorite, mercuric chloride, tincture of metaphen and Roccal. The germicidal action of sodium hypochlorite is due to an oxidation reaction. The amount of available chlorine in the sample, 440 p.p.m., was no indication of the amount of free chlorine residual remaining for virucidal activity after the chlorine demands of the organic

matter had been satisfied. That small amounts of free chlorine residual are virucidal has been reported by Ridenour and Ingols (1941) who showed that 0.2 p.p.m. free chlorine residual inactivated a 1:500 dilution of poliomyelitis virus after 10 minutes, or 0.1 p.p.m. after 30 minutes. Lensen, Rhian and Stebbins (1947), using poliomyelitis virus partially purified by ultracentrifugation and testing for free chlorine and chloramine, found that a 0.5 per cent suspension of the virus was inactivated after 10 minutes with 0.05 p.p.m. free chlorine residual if the pH was near neutrality. Their experiments suggested a decrease of virucidal action of the chlorine with increasing alkalinity. According to Stokes (1946), a free chlorine residual of one p.p.m. was sufficient to inactivate infectious hepatitis virus in 30 minutes.

The results obtained in the present studies with sodium hypochlorite, 440 p.p.m. available chlorine, indicate the effect of organic matter on the virucidal activity of the chemical agent. In the 1:1 mixtures at 0 C and 20 C, there was no detectable inactivation of NDV. In the 1:9 mixtures at the same temperature, the virus was inactivated. This would indicate that in the 1:1 mixtures there was no free chlorine residual for action with the virus but in the 1:9 mixtures, which contained five times more sodium hypochlorite than the 1:1 mixtures, there was sufficient free chlorine residual present to inactivate the virus.

Mercurial compounds are bacteriostatic rather than bactericidal. The effect of these compounds is probably due to adsorption of mercury ions to the surface of the bacterium with a resulting inhibition of metabolism. Following removal or neutralization of the ions, the bacterium may resume its metabolic activities. Precipitation of extraneous protein

material by the mercurial compounds would reduce the concentration of mercury available for adsorption to the bacterium. Assuming that the action of mercurial compounds on NDV is similar to that on bacteria, the extraneous protein material in the virus preparation probably influenced the action of the mercury ions in inactivating the virus. There was no detectable inactivation of NDV in the undiluted preparation in the 1:1 or 1:9 mixtures with mercuric chloride, 0.01 per cent, at 0 C, 20 C and 37 C for 120 minutes, but the virus in a 10^{-4} dilution in the 1:9 mixture at 20 C for 15 minutes was completely inactivated. This would indicate that, in the undiluted virus preparation, the mercury was probably adsorbed to the protein material and was unable to inactivate the virus. In the 10^{-4} dilution of the virus preparation, the protein material was probably reduced to a concentration that did not affect the action of the mercury ions on the virus. The results with tincture of metaphen would indicate that the concentration employed was too low to produce any appreciable effect on either the undiluted virus preparation or on the 10^{-6} dilution of the preparation.

With Roccal, which is adsorbed to the surface of a bacterium and probably also to a virus, the extraneous protein material probably did not have any appreciable effect on its virucidal properties.

The germicidal activity of phenol, ethyl alcohol and sodium hydroxide is not seriously impaired by the presence of small amounts of extraneous organic matter. Phenol and ethyl alcohol exert their action as non-ionized molecules. The effect of sodium hydroxide is due to the hydroxyl ions.

VI

SUMMARY

Studies of the effect of phenol, one per cent, ethyl alcohol, 40 per cent, sodium hydroxide, one per cent; sodium hypochlorite, 440 p.p.m. available chlorine; Roccal, 0.01 per cent; mercuric chloride, 0.01 per cent; and tincture of metaphen, 0.01 per cent, on Newcastle disease virus in proportions of virus:chemical agent of 1:1 and 1:9 at 0 C, 20 C and 37 C for different time intervals indicated a logarithmic decrease in viral activity when undiluted virus-infected allantoic fluid, 0.54 mg nitrogen per ml, l.d.50 $10^{8.8}$ per 0.1 ml, was employed. These findings were substantiated when the chemical agents were tested against different concentrations of the virus at 20 C for 15 minutes.

The initial stage of inactivation of the virus was detected earlier with the 1:9 mixtures than with the 1:1 mixtures. The rate of inactivation for the 1:9 mixtures was greater than that for the 1:1 mixtures with one exception with phenol, one per cent, where the reverse occurred.

Increased temperature augmented the action of ethyl alcohol, sodium hydroxide and sodium hypochlorite. Sodium hydroxide was also efficacious at low temperatures.

These studies showed that the number of infective doses of virus, the ratio of virus to chemical agent, the temperature of exposure, and the period of exposure exert an influence on the evaluation of virucidal tests. Extraneous protein material in the virus preparation may affect the action of oxidizing agents and adsorbing compounds.

VII

BIBLIOGRAPHY

Anderson, S. G. A note on two laboratory infections with the virus of Newcastle disease of fowls. Med. J. of Australia. 1:371. 1946.

_____ The reaction between red cells and viruses of the influenza group. Studies with Newcastle disease virus. Australian J. Exp. Biol. & Med. Sci. 25:163-174. 1947.

Asplin, F. D. Observations on the viability of Newcastle disease virus. Vet. Rec. 65:159-160. 1949.

Association of Official Agricultural Chemists. Official Methods of Analysis of the Association of Official Agricultural Chemists. Association of Official Agricultural Chemists. Washington, D. C. 1950.

Bang, F. B. Studies on Newcastle disease virus. I. An evaluation of the method of titration. J. Exp. Med. 88:233-240. 1948a.

_____ Studies on Newcastle disease virus. II. Behavior of the virus in the embryo. J. Exp. Med. 88:241-249. 1948b.

_____ Studies on Newcastle disease virus. III. Characters of the virus itself with particular references to electron microscopy. J. Exp. Med. 88:251-266. 1948c.

_____ Formation of filamentous forms of Newcastle disease virus in hypertonic concentration of sodium chloride. Proc. Soc. Exp. Biol. & Med. 71:50-52. 1949.

Beach, J. R. A nervous disorder in chickens. Nu-Laid News. 18:15. 1941.

_____ Avian pneumoencephalitis. Proc. 46th An. Meet. U. S. Livestock San Assn. 202-223. 1942.

_____ The neutralization <u>in vitro</u> of avian pneumoencephalitis virus by Newcastle disease immune serum. Science. 100:361-362. 1944.

Beamer, P. D. and J. E. Prier. Studies on Newcastle disease. III. Resistance of Newcastle disease virus to certain chemical agents. Cornell Vet. 40:57-60. 1950.

Beaudette, F. R. A review of the literature on Newcastle disease. Proc. 47th An. Meet. U. S. Livestock San. Assn. 122-177. 1943.

_____ An addendum to a review of the literature on Newcastle disease. Proc. 53rd. An. Meet. U. S. Livestock San. Assn. 202-220. 1949.

_____ Recent literature on Newcastle disease. Proc. 54th An. Meet. U. S. Livestock San. Assn. 132-153. 1950.

_____, J. A. Bivins, B. R. Miller, C. B. Hudson and J. J. Black. A comparison of filtration and antibiotic treatment for the recovery of Newcastle disease virus from spontaneous cases. Am. J. Vet. Res. 10:92-95. 1949.

Bivins, J. A., B. R. Miller, and F. R. Beaudette. Search for virus in eggs laid during recovery postinoculation with Newcastle disease virus. Am. J. Vet. Res. 11:426-427. 1950.

Brandly, C. A. Newcastle disease. J.A.V.M.A. 116:139-146. 1950.

_____, H. E. Moses, E. L. Jungherr and E. E. Jones. Epizootology of Newcastle disease of poultry. Am. J. Vet. Res. 7:243-249. 1946a.

_____ Isolation and identification of Newcastle disease virus. Am. J. Vet. Res. 7:289-306. 1946b.

_____ Transmission of antiviral activity via the egg and the role of congenital passive immunity to Newcastle disease in chickens. Am. J. Vet. Res. 7:333-342. 1946c.

Burnet, F. M. and J. D. Ferry. The differentiation of the viruses of fowl plague and Newcastle disease: Experiments using the technique of chorioallantoic membrane inoculation of the developing egg. Brit. J. Exp. Path. 15:56-64. 1934.

_____ The affinity of Newcastle disease virus to the influenza group. Australian J. Exp. Biol. & Med. Sci. 20:81-88. 1942.

_____ "Human infection" with the virus of Newcastle disease of fowls. Med. J. of Australia. 2:313-314. 1943.

Cunha, R., M. L. Weil, D. Beard, A. R. Taylor, D. G. Sharp and J. W. Beard. Purification and characters of the Newcastle disease virus. J. Imm. 55:69-89. 1947.

Cunningham, C. H. The effect of certain chemical agents on the virus of Newcastle disease of chickens. Am. J. Vet. Res. 9:195-197. 1948.

_____ Newcastle disease and infectious bronchitis neutralizing antibody indexes of normal chicken serum. Am. J. Vet. Res. 12:129-133. 1951.

_____ A laboratory guide in virology. 2nd ed. Burgess Publishing Co., Minneapolis. 1952a.

_____. Methods employed in the diagnosis and investigation of infectious bronchitis and Newcastle disease. Proc. 89th An. Meet. A.V.M.A. 250-258. 1952b.

DeLay, P. D. Isolation of avian pneumoencephalitis (Newcastle disease) virus from the yolk sac of four-day-old chicks, embryos, and infertile eggs. Science 106:546. 1947.

_____, K. B. De Ome and R. A. Bankowski. Recovery of pneumoencephalitis (Newcastle) virus from the air of poultry houses containing infected birds. Science. 107:474-475. 1948.

Doyle, T. M. A hitherto unrecorded disease of fowls due to a filter-passing virus. J. Comp. Path. Therap. 40:144-169. 1927.

_____ The virus diseases of animals with special reference to those of poultry. J. Comp. Path. Therap. 46:90-107. 1933.

Durusan, R. Thermal inactivation of Newcastle disease virus. Unpublished M.S. thesis. Michigan State College. 1949.

Evans, A. S. Serological studies on infectious mononucleosis and viral hepatitis with human erythrocytes modified by different strains of Newcastle disease virus. J. Imm. 64:411-420. 1950.

Farinas, E. C. Avian pest; a disease of birds hitherto unknown in the Philippine Islands. Philippine Jour. Agr. 1:311-366. 1930.

Florman, A. L. Some alterations in chicken erythrocytes which follow treatment with influenza and Newcastle disease virus. J. Bact. 55:183-196. 1948.

Gustafson, D. P. and H. E. Moses. Isolation of Newcastle disease virus from the eye of a human being. J.A.V.M.A. 118:1-2. 1951.

Hanson, R. P., N. S. Winslow and C. A. Brandly. Influence of the route of inoculation of Newcastle disease virus infection of the embryonating egg. Am. J. Vet. Res. 8:416-420. 1947.

_____, E. Upton, C. A. Brandly and N. S. Winslow. Heat stability of hemagglutinin of various strains of Newcastle disease virus. Proc. Soc. Exp. Biol. & Med. 70:283-287. 1949.

_____, N. S. Winslow, C. A. Brandly and E. Upton. The antiviral activity of Newcastle disease immune sera. J. Bact. 60:557-560. 1950.

Hirst, G. K. Receptor destruction by viruses of the mumps-NDV-influenza group. J. Exp. Med. 91:161-175. 1950a.

_____ The relationship of the receptors of a new strain of virus to those of the mumps-NDV-influenza group. J. Exp. Med. 177-184. 1950b.

Hofstad, M. S. A study on the epizootology of Newcastle disease (pneumoencephalitis). Poul. Sci. 28:530-533. 1949.

_____ A quantitative study of Newcastle disease virus in tissues of infected chickens. Am. J. Vet. Res. 12:334-339. 1951.

Holmes, F. O. The filterable viruses. In Bergey's Manual of Determinative Bacteriology. 6th ed. The Williams & Wilkins Co., Baltimore. 1948.

Hunter, M. C., A. H. Keeney and M. M. Sigel. Laboratory aspects of an infection with Newcastle disease virus in man. J. Inf. Dis. 88: 272-277. 1951.

Ingalls, W. L. and A. Mahoney. Isolation of the virus of Newcastle disease from human beings. Am. J. Pub. Health. 39:737-740. 1949.

Jungherr, E., E. E. Tyzzer, C. A. Brandly and H. E. Moses. The comparative pathology of fowl plague and Newcastle disease. Am. J. Vet. Res. 7:250-288. 1946.

_____, R. E. Luginbuhl and L. Kilham. Serologic relationship of mumps and Newcastle disease. Science. 110:333-334. 1949.

Keeney, A. H. and M. C. Hunter. Human infection with the Newcastle virus of fowls. A.M.A., Arch. Ophtham. 44:573-580. 1950.

Kilham, L. A Newcastle disease virus (NDV) hemolysin. Proc. Soc. Exp. Biol. & Med. 71:63-66. 1949.

_____ Evaluation of the agglutination of erythrocytes sensitized by Newcastle disease virus (NDV) as a serologic test in infectious mononucleosis. J. Imm. 65:245-253. 1950.

Kraneveld, F. C. Over een in Ned.-Indie heerschende Ziekte onder het Pluimvee. Nederlandsch-Indische Bladen voor Diergeneeskunde. 38: 448-450. 1926. Cited by Beaudette, 1943.

Lensen, S. G., M. Rhian and M. R. Stebbins. Inactivation of partially purified poliomyelitis virus in water by chlorination. II. Am. J. Pub. Health. 37:869-874. 1947.

McCulloch, E. C. Disinfection and sterilization. 2nd ed. Lea & Febiger. Philadelphia. 1945.

Moses, H. E. Report of the committee on Newcastle virus properties. J.A.V.M.A. 112:126-127. 1948.

_____, C. A. Brandly and E. E. Jones. The pH stability of viruses of Newcastle disease and fowl plague. Science. 105:477-479. 1947.

Prier, J. E., T. W. Millen and J. O. Alberts. Studies on Newcastle disease. IV. The presence of Newcastle disease virus in eggs of hens vaccinated with live vaccine. J.A.V.M.A. 116:54-55. 1950.

Reed, L. J. and H. Muench. A simple method of fifty per cent end-points. Am. J. Hyg. 27:493-497. 1938.

Reagan, R. L., M. G. Lillie, J. E. Hauser and A. L. Brueckner. Electron micrographs of the hamster-adapted Newcastle virus. Cornell Vet. 38:418-420. 1948.

Ridenour, G. M. and R. S. Ingols. Inactivation of poliomyelitis virus by "free" chlorine. Am. J. Pub. Health. 36:639-644. 1946.

Romanoff, A. L. Membrane growth and function. In The chick embryo in biological research. Ann. N. Y. Acad. Sci. 55:288-301. 1952.

Schmittle, S. C. Studies on Newcastle disease. V. A comparison of Newcastle disease antibody titers in blood and egg as measured by the hemagglutination-inhibition test. Am. J. Vet. Res. 11:226-230. 1950.

_____, and T. W. Millen. Studies on Newcastle disease. I. Detection of hemagglutination-inhibition antibodies in unincubated eggs. Cornell Vet. 38:306-309. 1948.

Stokes, J., Jr. Report of the Commission on Measles and Mumps. Hepatitis. Epidemiological Board, U. S. Army. 5. July 1, 1945-, March 15, 1946.

Stover, D. E. A filterable virus, the cause of a respiratory-nervous disorder of chickens. Am. J. Vet. Res. 3:207-213. 1942a.

_____ Respiratory-nervous disorder in eight-months old pullets. Am. J. Vet. Res. 3:239-241. 1942b.

Thompson, Jr., C. H. Newcastle disease infection in man. Military Surg. 106:276-281. 1950.

Tilley, F. W. and W. A. Anderson. Germicidal action of certain chemicals on the virus of Newcastle disease (avian pneumoencephalitis). Vet. Med. 42:229-230. 1947.

U. S. Department of Agriculture. The diagnosis of Newcastle disease. Mimeo. Circular. Washington, D. C. Aug. 15. 1946a.

_____. The hemagglutination and hemagglutination-inhibition tests for the diagnosis of Newcastle disease. Mimeo. Circular. Washington, D. C. Oct. 21. 1946b.

Winslow, N. S., R. P. Hanson, E. Upton and C. A. Brandly. Agglutination of mammalian erythrocytes by Newcastle disease virus. Proc. Soc. Exp. Biol. & Med. 74:174-178. 1950.