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AN EXPERIMENTAL STUDY OF THE
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AN EXPERIMENTAL STUDY OF THE PATHOGENESIS OF FOWLPOX
INFECTION IN CHICKENS

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the Graduate
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By

Ahmet Minbay, DVM, M.Sc.

* * * * *

The Ohio State University
1969

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I. INTRODUCTION

Fowlpox, which has been recognized as a disease entity for many centuries (Goodpasture, 1928; Beaudette, 1941, 1949; Sevoian, 1960), is characterized by the appearance of eruptive skin lesions. Spontaneous fowlpox nodules generally appear on unfeathered parts of the chicken's body, although in severe cases, typical lesions may develop on other parts of the body particularly around the cloacal aperture, the under-surface of the wings and on the neck. Sometimes lesions develop on the rump also (Hutyra, et al., 1946). Fowlpox is commonly seen in domestic fowl, frequently attacking an entire flock, when once introduced, and sometimes causing high mortality. The disease is endemic in many countries and frequently occurs in epidemic form if poultry is raised in such areas without vaccination (Beaudette, 1949).

Three clinical forms of fowlpox are recognized: In the common type which characterizes most epidemics, wart-like nodules develop on the comb, wattles and skin of the head; in the diptheroid type adherent, yellow cheesey membranes form in the mouth; in the type which is referred to as "roup" a watery or mucupoulent liquid is discharged from the eyes and nose. These forms may occur alone or in any combination in

the same bird (Doyle and Minett, 1927, Hutyra, et al, 1946, Beaudette, 1949).

The pathogenesis of fowlpox is still not completely understood. The clinical form of fowlpox is not easily produced in the laboratory except by the highly artificial means of intravenous injection. We hoped that information on the sites of growth of strains of fowlpox virus of differing pathogenicity, after introduction by various routes, would aid us in gaining an understanding of the pathogenesis of fowlpox.

The specific objectives of this study were to determine in which organs the fowlpoxvirus multiplies and to determine the sequence of spread of the virus through the body following intravenous, intradermal and intratracheal inoculations. Special attention was paid to the differences in the pathogenesis of fowlpox in chickens infected with different strains of virus.

Complete understanding of the pathogenesis will probably only follow studies similar to these but carried out on chickens involved in natural outbreaks. Such studies lie in the future.

II. LITERATURE REVIEW

A. General

Fowlpox virus is the largest of the animal viruses. The virus particles can be seen with phase optics (Angstrom, 1951; Herzberg and Kleinschmidt, 1959) or in stained preparations examined with the light microscope (Marx and Sticker, 1908; Angstrom, 1951; Beaudette, 1949; Goodpasture, 1928). In stained preparations examined by light microscopy, the viruses appear as small spherical particles. In electron micrographs the virus particles appear to have six rectangular surfaces so arranged that their three dimensional shape is brick-like (Groups, et al. , 1947; Morgan, et al. , 1954) or they appear as flattened oval bodies, the axes of which have the ratio 1.4:1.8:3.0 (Herzberg and Kleinschmidt, 1959). The virus particles have a complex structure consisting of an internal central mass, the nucleoid, surrounded by two membrane layers. In shadowed preparations, the average dimensions of the particles were reported to be 258 mu by 334 mu. In preparations stained with phosphotungstic acid (PTA) the particles appeared to be 261 mu by 354 mu (Randall, et al. , 1964).

Fowlpox virus is of the deoxyribonucleic acid (DNA) type, the DNA of which can be shown to contain 35% guanine and cytosine by either

chemical (Randall, et al. , 1962) or physicochemical methods (Szybalski, et al. , 1963). Fowlpox virus (Poxvirus avium) is a member of the pox-virus group which also includes smallpox (P. variolae), vaccinia virus (P. officinale), cowpox virus (P. bovis), Ectromelia (P. Muris), and myxoma virus (P. myxomatis) (Fenner and Burnet, 1957). Other viruses belonging to the pox group include molluscum contagiosum, goat pox, sheep pox, contagious pustular dermatitis (P. orfi), bovine papular stomatitis, monkey pox and fish pox (Fenner and Burnet, 1957).

Fowlpox virus is stable, it has been shown to survive in the dried state at room temperature for about a year (Beaudette, et al. , 1948). The virus is inactivated by heating for twenty minutes at 50°C., or eight minutes at 60°C., (Fenner, 1958) and is resistant to ether but sensitive to chloroform although both these agents are fat solvents. The fowlpox virus withstands treatment with phenol diluted 1:100 or formalin for nine days (Andrewes, 1964). The antigens of fowlpox virus are composed of a complex of proteins in which at least two components are recognized; these are soluble or LS antigen (Craigie and Wishart, 1936) and nucleoprotein (NP) antigen (Smadel, et al. , 1942). It has not been possible to demonstrate a hemagglutinin in association with fowlpox virus in suspensions of heavily infected chorioallantoic membranes, using the technique by which the hemagglutinin of the mamalian pox viruses was demonstrated (Fenner and Burnet, 1957). A passive hemagglutination test, using tanned horse red blood cells

coated with partially-purified fowlpox was reported for titration of antibodies against fowlpox virus (Tripathy, 1967). The "NP" antigen appears to contain a group antigen common to all viruses of the pox-virus group (Woodroffe and Fenner, 1962).

There appear to be at least four different viruses or strains of virus causing pox among birds. These are classified by Van Rooyen (1954) as Borreliota avium (fowl poxvirus), Borreliota meleangridis (turkey poxvirus), Borreliota fringillae (canary poxvirus) and Borreliota columbae (pigeon poxvirus). Each virus is infective for its homologous host and in some instances for heterologous hosts. Various degrees of cross protection are conferred on birds by infection by various avian pox viruses (Beaudette, 1941, Seeger and Price, 1956) and cross-neutralization between canary pox and fowlpox viruses has been demonstrated on the chorioallantois, indicating that canary pox and fowlpox are serologically similar (Burnet and Lush, 1936).

Knowledge of the pathogenesis of fowlpox is derived from clinical and pathological studies in naturally infected chickens and from laboratory investigations of experimentally infected birds. Chickens of all ages, sexes and breeds are equally susceptible to the infection (Beaudette, 1929, Johnson, 1938). The incubation period of the spontaneous disease varies from 8 to 14 days (Goodpasteur, 1928, Delaphane, 1943). The course of the uncomplicated disease is usually 3 to 4 weeks. Recovered birds are immune to subsequent infection.

Fowlpox virus is unable to penetrate intact epithelium. Infection is believed to result from injuries sustained in pecking and mouth lesions are believed to result from injuries produced by eating of sharp grit (Doyle and Minett, 1927). Francis (1956) reported the possibility of airborne infection and mosquitoes have also been incriminated in transmission of the disease (Seddon, 1952, Kligler and Ascher, 1929, Francis, 1956). It is generally believed that the virus after entering the skin or mucous membranes multiplies first in epithelial cells. The virus appears in the blood within 24 hours after entering the skin (Francis, 1956, Dhillon, 1966) and is carried to other parts of the skin and mucous membranes where it causes the formation of specific pox eruptions. The virus has been demonstrated in the internal organs including the liver, kidney, heart and muscle (Doyle and Minett, 1927) and in the lungs (Francis, 1956).

The possibility that "carrier chickens" serve as foci of fowlpox infection has been the subject of considerable research work. Doyle and Minett (1927) indicate that there is no evidence that the carrier state persists for any long period of time after the disappearance of the pox lesion.

Dhillon (1966) studied the persistence of fowl poxvirus in the blood of chickens following intravenous and intradermal inoculation. Dhillon reported that most of the virus was removed from the blood within 15 minutes following intravenous inoculation and the blood was essentially free of virus

within 4 hours. The virus particles again reappeared in the blood by the fifth day and persisted in the blood in small amounts for 20 to 25 days.

B. Pathogenesis

Fowlpox occurs commonly in chickens, turkeys, guinea fowl, pigeons and canaries; more rarely in ducks, geese, pheasants, quail and hawks. The virus differs somewhat in pathogenicity for different species and is usually more pathogenic for the host from which it was recovered than for heterologous hosts. Some fowl poxviruses may be mono-, bi-, or tripathogenic with respect to transmission to certain species of birds (Reis and Nobrega, 1937; Siegel, 1957).

Avian poxviruses are commonly considered to fall into four groups: fowl poxviruses, turkey poxviruses, canary poxviruses and pigeon poxviruses (Van Rooyen, 1954). Beach (1939) compiled a list of susceptible species including those which acquire the disease naturally and those which have been infected experimentally. The interested reader is referred to his paper. Fowl poxvirus is infective for chickens of all ages, sexes and breeds (Goodpasture, 1928; Beaudette, 1929). Turkeys (Pilchard, et al., 1962; Brunett, 1934; Brandly and Dunlop, 1938) and quail (Gallagher, 1916) are susceptible to natural infection; pigeons (Doyle and Minett, 1927) and

canaries (Burnet, 1933) are reported to be susceptible to experimental infection. Turkey poxvirus is infective only for turkeys under natural conditions (Irons, 1924). Brandly and Dunlap (1938) reported that some strains of turkey pox are infectious for chickens experimentally, and Beaudette and Hudson (1941) using a strain of turkey poxvirus produced more severe local and systemic reactions and a higher percentage of secondary head lesions in chickens than they did with fowl poxvirus. Turkey poxvirus, like fowl poxvirus, is not naturally infective for pigeons and artificial transmission to the pigeon is difficult (Brunett, 1934).

Pigeon virus is naturally infective for pigeons and chickens. Pigeon poxvirus produces only a swelling of the follicles, and remains localized in the chicken. Following infection with pigeon poxvirus chickens are immune to fowl poxvirus (Beaudette, 1949). Turkeys (Brunett, 1934), canaries (Irons, 1934) and sparrows (Burnet, 1933) are susceptible experimentally to canary pox. There appear to be at least three different strains of virus causing pox in canaries. Kikuth and Gallup (1932) reported the isolation of a canary poxvirus which is infective for canaries naturally and infective for sparrows and pigeons experimentally. The canary poxvirus isolated from canaries in 1937 by Reis and Nobrega was found not to be infectious for chickens and pigeons. Reis and Nobrega (1936) also reported another strain of canary poxvirus which was a tripathogenic strain

infecting chickens, pigeons and canaries. Canary poxvirus produces a disease with high mortality in canaries. The disease caused by canary poxvirus in chickens, pigeons and sparrows is local and recovery is spontaneous.

Avian poxviruses may cause localized or generalized, acute or chronic, mild or fatal diseases in susceptible birds. It is apparent that the more virulent strains of avian poxviruses show a more or less uniform pattern in their pathogenesis. Goodpasture (1928) and Huttyra and Marek (1946) give excellent accounts of the pathogenesis of spontaneous pox in chickens. Little has been added to our knowledge of the histology of the specific lesions on the skin and the mucous membranes since these early descriptions on the pathogenesis of fowlpox. Most of the work that has been done since that time merely confirms the earlier observations.

The incubation period of the spontaneous disease varies from 4 to 6 days (Goodpasture, 1928) to 8 to 14 days (Delaphane, 1943), depending upon the virulence of the infecting fowl poxvirus. After the incubation period the most characteristic clinical feature of the disease, the cutaneous eruption, appears usually upon the unfeathered parts of the body. The lesions are observed particularly about the head, affecting the comb, wattles, eyelids, nares and angle of the beak (Goodpasture, 1928, Huytra and

Marek, 1946). The lesions may also appear on the legs and feet (Stafseth, 1931). The cutaneous lesions are characteristically nodular and are about 1 mm in diameter when they are first seen. Later the nodules become enlarged, obtaining a diameter of about 0.5 cm. The pox lesions go through the typical stages of development of pox diseases: roseolar, popular, vesicular and pustular. The cutaneous nodules may be numerous or few in number. Because they do not necessarily erupt at the same time, all stages of development can be seen in the same bird at the same time (Hutyra and Marek, 1946).

The disease may be demonstrated experimentally by scarifying the comb or skin and placing the virus on the injured tissue (Goodpasture, 1928; Dhillon, 1966), by intravenous injection (Burnet, 1906; Dhillon, 1966), and by infection through the respiratory system (Francis, 1956). The only experimental procedure which reproduces exactly the picture of the natural disease is intravenous inoculations (Burnet, 1906; Dhillon, 1966).

The virus is abundant in the skin and mucosal lesions of diseased chickens (Angstrom, 1951; Beaudette, 1949; Pilchard, 1959). The virus may also at times be demonstrated in the blood stream (Doyle and Minett, 1927; Dhillon, 1966; Francis, 1956) and in the internal organs (Doyle and Minett, 1927; Francis, 1956). Some workers doubt that the virus attacks

any tissues other than the epithelium (Brunett, 1945). On the other hand, other workers, in spite of their failure to find fowl poxvirus in the blood or lymph, attributed the occurrence of disseminated and generalized forms of the disease eruption of the skin to the release of the virus particles from the local lesions where they are produced into the blood which disseminates it (Sanfelice, 1914).

On clinical (Goodpasture, 1928), histological and pathological (Hutyra and Marek, 1946) grounds it is believed that the fowl poxvirus enters the body through abrasions, and other minute cuts and wounds on the skin or mucous membranes which result from pecking and fighting. The virus particles multiply first in the epithelial cells at the site of entry (Sanfelice, 1914, Dhillon, 1966, Doyle and Minett, 1927, Beaudette, 1941). From the point of entry, following the multiplication, the virus enters the blood stream within 24 hours (Sanfelice, 1914, Dhillon, 1966). The virus is carried in the blood to other parts of the skin and mucous membranes where it causes the formation of secondary pox eruptions and also to the internal organs in which it multiplies and causes inflammation and cell degeneration (Sanfelice, 1914, Francis, 1956, Dhillon, 1966).

The persistence of the virus in the blood and the multiplication of fowl poxvirus in the internal organs have not been thoroughly studied. There is

every reason to believe that fowl poxvirus is present in the circulating blood at some period during the course of the disease, but information of the sequence of the events is fragmentary. Direct evidence on the pathogenesis of fowlpox is generally not available and the pathogenesis of fowlpox infection is commonly based upon information provided by the study of other poxvirus infections in other animals.

In the well known classical experimental work on pathogenesis of ectromelia, Fenner (1948) described the microscopic and histologic appearance of the skin lesions of mouse pox (ectromelia) and the pathogenesis of the disease. Groups of mice were inoculated in the foot pads with small amounts of a suspension of ectromelia virus containing infective particles. Multiplication of ectromelia virus was determined by daily titration of virus in samples taken from the foot, spleen, blood and skin of infected mice. Fenner reported that following the inoculation of the ectromelia virus into the skin, the virus undergoes a step-by-step invasion of the body. The regional lymph nodes are invaded, then the liver and spleen; multiplication of the virus in these organs caused a secondary viremia during which multiple foci of infection in the skin resulted in the development of the rash some days after the inoculation of virus. Many writers have accepted this sequence of events as a model for the pathogenesis of all pox virus infections.

Fenner and Woodroffe (1953) studied the course of myxomatosis infection in European rabbits. They noted that the pathogenity of myxomatosis is similar to that of the mousepox studied earlier by Fenner. According to their work, myxoma virus spreads in a systematic fashion from the site of inoculation, through the local lymph node and blood to the internal organs where the myxoma virus, probably multiplies in the walls of small arterioles (Hurst, 1937). The viremia which develops after growth in the internal organs enables the virus to reach the skin and further multiplication there gives rise to the generalized lesions. The virus is widely distributed throughout the tissues of the body and is especially abundant in the swollen tissues and the lymph nodes. The steady rise in titer of virus in all affected organs indicates the multiplication of the myxoma virus in those organs. Myxoma virus has been shown to be present in the white cell fraction of the blood.

Bedson and Duckworth (1963) found that infection with rabbitpox in rabbits follows a sequence similar to that of ectromelia in mice reported by Fenner (1948). The spread of virus within the body was traced in rabbits infected by intradermal inoculation of rabbit poxvirus. Bedson and Duckworth reported that the progressive stepwise invasion by the virus involves first the primary site of infection, then the regional lymph nodes, and then

the spleen and other remote tissues. Relatively low titres of virus were found in the blood and virus was not demonstrated there until after extensive dissemination of virus had occurred. Bedson and Duckworth also reported that virus in the blood was principally associated with the buffy coat.

Somewhat similar findings have been reported in an experimental study of variola infection in rhesus monkeys (Hahon and Wilson, 1960). It was reported that there was extensive multiplication in the lungs following infection by inhalation of a small particle aerosol. At the height of proliferation in the lungs or even earlier the virus was found in the blood.

C. Transmission

The virus is found to be abundant in the lesions of fowlpox in the skin and mucous membranes. From the infected host, fowl poxviruses are discharged into the environment from the lesions on the skin and mucous membranes. Infection of new hosts is believed to occur in most instances directly or indirectly through the skin or exposed mucous membranes. The fowl poxvirus is unable to pass through the undamaged skin and mucous membranes. Doyle and Minett (1927) reported that they failed to produce infection or confer immunity in chickens by daily

applications of virus material to the unbroken surface of the comb over a period of 6 days. They also reported that feeding gelatin capsules containing virus did not produce disease in chickens. Burnet (1906) failed to infect a pigeon by giving the virus in the drinking water over a period of one month. In the absence of injury or insect transmitters the disease usually does not spread experimentally even with close contact. Because chickens often have numerous small injuries around the head and mouth as a result of continuous pecking and fighting, the disease spreads to susceptible birds kept in the same pen with diseased stock (Kossock, 1954). The disease condition may be successfully produced experimentally by scarifying the comb or skin and placing the virus on the injured tissue. (Doyle and Minett, 1927, Goodpasture, 1928, Dhillon, 1965.)

Typical fowlpox lesions appear at the site of inoculation 4-5 days after the viral material is introduced.

Fomites may be carriers of the virus from one bird to another. As early as 1906 Cary stated that mosquitoes and other insects may sometimes be carriers of fowlpox virus. Since that time a number of investigations (Kligler, et al., 1929, Kligler, 1930, Blanc and Caminopetros, 1930, Stuppy, 1932) have experimentally demonstrated that Aedes aegypti and Culex pipiens may transmit fowlpox from infected chickens. Mosquitoes

may produce a number of consecutive infections for at least 10 days and the virus may become localized on the proboscis of the mosquito, which may remain infective from 16 to 19 days (Kligler and Aschner, 1929). Infected Culex pipiens may transmit the infection for at least 39 days (Stuppy, 1932) or 58 days (Blanc and Caminopetros, 1930). Despite the long periods of infectivity of mosquitoes, most writers believe that mosquitoes are just mechanical transmitters of the disease (Francis, 1956). Broody (1936) reported that Adedes aegypti can definitely transmit fowlpox more than once during its life, starting to transmit within one hour after the blood meal and transmitting through the 59th day. He also indicated that mosquitoes must feed through a pox lesion where the virus is abundant in order to become contaminated with the virus. It appears that in Australia mosquitoes are largely responsible for the spread of fowlpox (Seddon, 1952).

Broody (1936) reported that the disease was transmitted experimentally by the northern fowl mites (Liponyssus sylviarum). He collected mites which had fed on a bird with fowlpox, killed them by grinding and suspended the resultant mite homogenate in water. A chicken in which this suspension was applied to the scarified skin developed typical fowlpox lesions.

The survival of the virus in dried exudate or in dust may, in some instances, facilitate infection by inhalation. Francis (1956) reported

experimental work in which only occasional birds developed slight lesions following exposure to infected dust or mist. The exposure to dust or spray for 15 minutes or one hour produced a good immunity.

Investigators have discussed the possibility that "carrier birds" serve as foci of fowlpox infection since fowlpox virus has been demonstrated in internal organs. Sanfelice (1914) claimed that pigeon pox-virus may be localized in certain organs and may persist there for up to 71 days. Doyle and Minett (1927) attempted to establish the presence of the virus in various tissues by tracing the virus in samples taken from infected birds. Birds were killed in 4 to 41 days following the intradermal and intravenous inoculations. In no case could Doyle and Minett demonstrate the virus in the blood or internal organs in the absence of active lesions. They concluded that there was no evidence that a carrier state persisted for any considerable period after the disappearance of lesions. Francis (1956) reported virus multiplication in the lungs following exposure to airborne infective particles, but he considered that the lungs do not act as reservoirs of infection between seasons.

D. Cultivation and Titration

Fowl poxvirus, like other viruses, cannot grow outside living cells. Sources of living cells for fowl poxvirus growth are: susceptible birds,

embryonated hen's eggs and tissue cultures.

Animal inoculation was the first method of virus cultivation and for many years the only means of virus propagation. Animal inoculation with materials containing the virus was practiced by incision or puncture of the skin or mucous membranes of susceptible animals for active immunization purposes. Variolation, the inoculation of smallpox material on the scarified skin of man (Downie, 1964), the inoculation of foot and mouth disease material on scarified areas of buccal mucosa and tongue (Frankel, 1950), inoculation of ectromelia virus into the foot pads of mice (Fenner, 1948), and inoculation of myxomatosis virus on rabbit skin (Fenner and Woodroffe, 1953) are good examples of this type of cultivation. In birds, the pox lesion material without treatment or after the suspension of material is filtered, can be directly introduced into susceptible birds for diagnostic purposes. Antibiotics may be added to suspension of lesion, organs or exudates before they are used as inoculating material for diagnosis or titration. Findlay (1928) described the simplest method for inoculation of birds with fowl poxvirus. In his procedure the crude suspension of the material was prepared by grinding the fowl poxvirus lesions with sand and Locke's solution in a mortar. The crude suspension then was centrifuged lightly and the supernatant was filtered through a Berkefield candle filter. The filtered material produced fowlpox lesions. Since that time, many workers have employed similar procedures for the preparation of inoculating material.

Skin of susceptible birds is used for the enumeration of fowl pox-virus as well as for its primary isolation and identification in suspected material. Titration of virus on skin of susceptible birds has fallen into disuse, however, since the fowl poxvirus can more easily be propagated and titrated on chorioallantoic membranes (CAM) of embryonated eggs. Some investigators, working with other pox viruses, have reported that titration of virus on the skin of susceptible animals is more sensitive than are the other techniques (Fenner, 1948; Fenner and Woodroffe, 1953).

The study of viruses was greatly expanded during the 1930's. The increase in study of viruses was closely associated with the introduction of the use of the chicken embryo for the cultivation of viruses. Fowl poxvirus was the first virus to be studied systematically on the CAM. Woodruff and Goodpasture (1931) studied the histology of the lesions and inclusions caused by the fowl poxvirus infection. Burnet (1935) modified the original Goodpasture technique and introduced the method of dropping the CAM to provide more space for inoculation of measured quantities of inoculum. Techniques for dropping the CAM of embryonated eggs vary widely. The methods have been described by several workers (Beveridge and Burnet, 1946; Hahon, et al., 1957; Budding, 1949). Few workers outside Burnet's laboratory have been completely

satisfied with the results of titrations of fowl poxvirus on CAM by pock counting methods, nevertheless, pock counting has been the primary method used for titration of poxviruses in infected material (Fenner, 1946; Bedson and Duckworth, 1963; Fenner and Woodroffe, 1953; Dhillon, et al., 1968). It appears that irregularity in the results obtained by the drop membrane pock count technique experienced by some workers (Haagen and Crodel, 1937) is not caused by difficulty in interpretation or counting of the pocks developed on CAM but, that the main problem arises from the difficulty in obtaining uniform suspensions of the virus particles. Goodpasture and Woodruff (1931) failed to liberate fowl poxvirus particles by grinding the infected tissues in saline whereas the homogenous and uniform distribution of *Molluscum contagiosum* was accomplished relatively easily. On the other hand, Findlay (1928) was able to prepare a crude suspension of the material by grinding the fowlpox lesions with sand and mortar in Locke's solution.

The significant feature of fowlpox is the presence of intracytoplasmic inclusion bodies in the affected epithelial cells (Goodpasture, 1928; Sevoian, 1960). A one per cent solution of trypsin will completely digest the host cell material of the fowlpox lesion in about 30 minutes, leaving the inclusion bodies free and separable from the tissue debris (Goodpasture, et al., 1931; Randall and Gafford, 1960; Dhillon, 1966).

The inclusion bodies have an elastic, semi-permeable membrane of lipoprotein composition and respond to osmotic influences (Woodruff and Goodpasture, 1931). Woodruff and Goodpasture (1929) showed that a single inclusion body inoculated into the skin of susceptible chicken produces a typical fowlpox lesion and later Woodruff and Goodpasture (1929) demonstrated that an inclusion body may contain as many as 20,000 elementary bodies each of which is capable of inciting a pock lesion. In addition to the problem in titration, caused by grouping of the fowl poxvirus particles in inclusion bodies, problems arise because the elementary bodies tend to form aggregations even after they are freed from the inclusion bodies. The fact that typical pocks may result from a single virus particle, an inclusion body, or an aggregate of particles makes the accurate and reproducible titration of a sample almost impossible.

The suspension of the ground lesion in N-Z-Amine prepared in distilled water is reported to give homogenous dispersion of the fowl poxvirus (Dhillon, et al., 1968). The distilled water in this solution breaks up the inclusion bodies and the amino acids in the N-Z-Amine stabilize the suspension of dispersed virus particles and gives a uniform suspension of virus particles.

The use of ultrasonic energy to liberate the elementary bodies is another approach to the problem of suspending the virus in solution. Ultrasound has been used to effect the release of the virus from the CAM (Randall, et. al. , 1962; Dhillon, et al. , 1968) and it is reported that sonication of trypsin-liberated fowlpox viral inclusions increased the virus titer, but Bedson and Duckworth (1963) sonicated blood samples from rabbits infected with rabbit poxvirus and they reported that the procedure did not increase the virus titer.

It appears that the CAM of embryonated eggs is the tissue best suited for the titration of fowl poxvirus. Gorham (1957) described a simple procedure for the titration of fowl poxvirus. In this method, a single hole is made through the shell over the air sac of eggs incubated less than 12 days, and a drop of inoculum is deposited onto the shell membrane with a long 21-24 gauge needle and the needle is inserted through the drop and underlying membrane. This method was employed for routine titration of fowl poxvirus in the serum of experimentally infected turkeys (Pilchard, 1961; Pilchard, et al. , 1962). Working with partially purified suspensions and whole blood samples from intradermally and intravenously infected chickens, Dhillon, et al. , (1968) found that the drop membrane method for titration of fowlpox virus was more consistent, more effective and more sensitive than the simple method of

Gorham (1957). Epithelial cells of ectodermal origin have been shown to be much more sensitive to fowl poxvirus than endothelial cells (Woodruff and Goodpasture, 1930). In the simple method capillary forces move the inoculum fluid into the allantoic cavity of the developing egg through the hole at the inoculation point and only a few of the inoculated particles at the inoculation point are able to contact susceptible cells. In the drop membrane method, many more virus particles come into contact with the CAM and have an opportunity to infect the susceptible ectodermal epithelial cells of the chorion. Fowl poxvirus produces typical pock lesions on CAM's and pock forming units are determined by enumeration of pock lesions. The titration of fowl poxvirus by the pock count is analogous to plaque counting with bacteriophage (Delbruck, 1945) or poliomyelitis (Dulbecco and Vogt, 1954).

As early as 1928, Findlay tried to cultivate fowlpox virus in tissue cultures of skin. Many workers continued in the attempt to use tissue culture for the cultivation of fowl poxvirus. Brandly and Dunlap (1938); Bang, et al., (1951), employed several methods of tissue culture for the cultivation of pigeon pox and fowl poxvirus. A minced chick-embryo in tyrode solution gave the most satisfactory results. Although, Bang, et al., reported that continuous cultivation of virus was not difficult, one cannot find much literature confirming their statement. According to Buthela and

Mathews (1957), fowlpox virus proliferates in trypsinized chick embryo kidney cell cultures but causes no cytopathogenic effect. Kangude and Hanson (1960), reported that Dulbeco's plaque technique has been found unsuitable for quantitation of fowl poxvirus, for the pinpoint plaques produced by fowl poxvirus are not easily detected by the naked eye.

E. Determination of virus titre

Virus material is titrated to determine the smallest amount of virus suspension or smallest number of virus particles capable of producing an observable manifestation in the susceptible host. From this smallest amount of virus, the infectious unit, the amount of virus in a given preparation can be calculated. The multiplication of viruses in susceptible tissues is demonstrated by the increased number of virus particles in samples taken from the same tissue in time intervals.

The most common titration procedure is the inoculation of equal samples of successive dilutions of the virus preparation into the susceptible host. Fowl poxvirus titrations are usually achieved by determination of the "end point" in embryonated eggs or by the "pock count" method on CAM of embryonated eggs. The end point is determined by scoring each inoculation as positive or negative according to the response of the embryonated eggs (Parker and River, 1936). Reed and Muench (1938) modified this

method and introduced the cumulative fifty percent infectivity end point (ID 50). In this method the percent of positive manifestation is calculated not from the actual frequency for any dilution, but from the accumulated sum. The procedure is based on the assumption that if a given individual host, for example an egg, is positive at a certain dilution, it will be positive if used at any lower dilution and vice versa for negative results. The limitation of the end point technique is that only the end response in an individual is determined. The amount of virus required to bring this change is not measured. The best results in determining the end point could be obtained by the use of large numbers of eggs at dilution near the value for the 50 percent reaction.

Beveridge and Burnet (1946) described the pock count method for the titration of viruses. The pock count method has been used for the titration and determination of titers of some poxvirus successfully (Fenner, 1948; Fenner and Woodroffe, 1953; Hahon and Wilson, 1960; Bedson and Duckworth, 1963) although the technique has not been widely accepted for routine titration of fowlpox virus because the results obtained by this method have not been found reproducible (Haagen and Crodel, 1939). Dhillon (1966) reported the disappearance of fowl poxvirus from circulating blood in 4 hours titrating the virus particles in blood samples employing the pock count method. The titre of the original viral suspension was determined

by the formula $n = \frac{y}{vx}$ where n = pock forming units, y = number of pocks per membrane, v = volume of virus inoculated and x = dilution of the virus.

III. MATERIALS AND METHODS

A. Experimental design, routes of infection, virus strains and numbers and types of birds inoculated

All birds were inoculated when about 5 weeks old. White leghorn chickens were used in three sets of experiments in which methods of infection were studied:

Experiment I, followed closely the experiment described by Fenner (1948) in studies on mousepox. The intradermal route of inoculation was used. One group of 30 birds was inoculated on the previously scarified combs with 0.05 ml of undiluted (10% suspension of CAM) challenge strain virus. Another group of 30 birds was inoculated in the same manner with about the same amount of the vaccine strain virus. A third group of 10 birds was inoculated with 0.05 ml of 1:100 dilution of outbreak strain virus.

In experiment II, the intravenous route of infection was used, as several workers had reported that the only experimental procedure which reproduces exactly the picture of natural fowlpox is intravenous inoculation of chickens with fowlpox virus (Burnet, 1906; Dhillon, 1966). In experiment II, one group of 30 birds was inoculated with 0.5 ml of undiluted challenge strain virus. In these birds, the primary viremia was

produced artificially and the sequence of events was studied from this stage of the disease. A second group of 30 birds was inoculated intravenously with the same amount of undiluted vaccine strain virus for the study of comparative pathogenicity of these two strains.

In experiment III, the intratracheal route of infection was used as the lungs were reported to constitute the principal site of primary infection in rhesus monkeys exposed to aerosolized variola virus (Hahon and Wilson, 1960) and in chickens following the intravenous inoculation of fowlpox virus (Francis, 1956). In experiment III, one group of 30 chickens was inoculated intratracheally with 0.05 ml of challenge strain of virus.

In all experiments birds were observed daily to study the development of the disease. Two chickens from each group were challenged to determine the effectiveness of the infection; one each by the intradermal and one each by the intravenous inoculation of large doses of challenge or outbreak strain of virus.

One bird of each experimental group was killed daily and blood, liver, spleen, kidney, lungs, brain and combs collected and titrated for virus content. Comb samples, collected from the birds inoculated intradermally in the comb in experiment I, yielded information for plotting the growth curve of virus in primary lesions. The growth curve

of virus in secondary lesions was determined from titration of comb samples obtained from the birds infected intravenously in experiment II.

The virus content of the internal organs was determined by titration of the various samples collected from the birds killed at daily intervals in each experimental group. Experiment III was specifically designed to study the course of virus spread in chickens after intratracheal inoculation.

In experiment I, a search was made for the development of a viremia after intradermal inoculation. In experiment II, the persistence of the viremia after intravenous inoculation and the occurrence of a secondary viremia was studied. In poxvirus infections including vaccinia in rabbits (Smith, 1929), myxomatosis (Fenner and Woodroffe, 1953), rabbitpox (Bedson and Duckworth, 1963) and smallpox in monkeys (Hahon and Wilson, 1960) virus has been shown to be present in the white cell fraction of the blood. During the secondary viremia stage in fowlpox, Dhillon (1966) reported small quantities of virus in blood. Experiments were, therefore, made to determine the distribution of virus in blood. Heparinized blood was separated by centrifugation into three fractions; plasma, buffy coat and red cells. In order to detect the small quantities of virus in the various fractions of blood, samples of 0.25 ml of undiluted plasma 1:10 dilutions of buffy coat and 1:5 dilutions of red cells in N-Z-Amine solution were inoculated on CAM's embryonated eggs.

Dhillon (1966) reported that virus disappears from the circulations of the intravenously infected chickens in a very short time, therefore, an individual chicken from the group of intravenously infected chickens was killed at hourly intervals or less. Samples were collected to determine the site of initial localization of virus in the internal organs. The first samples were collected from birds killed at 0 time (right after the injection of virus), at 15 minutes and at 30 minutes. One chicken was killed then every hour for 24 hours post inoculation, except that no samples were collected at the 18th, 20th and 22nd hours. Two groups of birds were used for this experiment; one group of birds was inoculated with challenge strain and one with vaccine strain virus.

B. Materials

1. Strains of fowl poxvirus: Three strains of fowl poxvirus were used in this study. The Vaccine (Va) virus and the Challenge (Ch) virus were kindly supplied by Dr. T. M. Goldhaft of Vineland Poultry Laboratories, Vineland, New Jersey. The Va virus is used as a fowlpox vaccine which is prepared by the Vineland Laboratories. Though the virus is modified and does not now produce viremia when intradermally introduced, many years ago, when it was first isolated, the strain produced secondary lesions at points other than the site of inoculation (Goldhaft, 1969). The

strain was maintained by chorioallantoic membrane (CAM) passage. The Ch strain has been used as a challenge virus in the Vineland Laboratories and has been previously studied in our laboratories (Dhillon, et al., 1968). The Ch strain produces lesions in birds and pocks on CAM of developing chick embryos. Dhillon (1966) reported the presence of virus in blood 24 hours after intradermal inoculations of the challenge strain and the development of secondary lesions. Lyophilized samples of the strain were kept in the freezer until used in this study. The Outbreak (Ou) strains were kindly supplied by Dr. Otto Schwabe of the New Jersey State Poultry Diagnostic Laboratory. The materials were collected from several outbreaks of natural fowlpox in the fall of 1968. The Ou strains were supplied as frozen CAM from eggs inoculated with the scab materials. The Ou strains were passaged on CAM of embryonated eggs only once and stored in frozen state until used.

2. Source of eggs and experimental birds: Fertile eggs were obtained through the courtesy of the Poultry Science Department of the Ohio State University. The layers at the department farm were known to be free from fowlpox and had not been vaccinated against fowlpox.

The birds used for experimental injections were procured from the Dekalb Marion Hatchery, 2701 New Road, Marion, Ohio. The day-old white leghorn cockerels received from that farm were kept isolated from

birds experimentally infected with fowlpox virus until use. The birds were used for experiments when they were 4-5 weeks old.

3. N-Z-Amine solution: A 10% solution of N-Z-Amine prepared in double-distilled demineralized water was used as virus diluent in all experiments. N-Z-Amine type AS, which is a pancreate digest of casein, was obtained from the Sheffield Chemical Company, Norwich, New York. The 10% solution was sterilized by autoclaving at 15 pounds pressure for 30 minutes and stored in a refrigerator until used. This diluent solution was used as solvent and dispersing agent for fowlpox virus. One milliliter of stock antibiotic solution, containing 100,000 units of penicillin and 10,000 ugm of streptomycin per ml was added to each 100 ml of N-Z-Amine solution so that the working diluent contained a final concentration of 1,000 units of penicillin and 100 ugm streptomycin per ml.

4. Heparin solution: A sterile neutral solution of heparin (Cannought Medical Research Laboratories, Toronto, Canada), 1,000 international units per ml, was used as anticoagulant for blood samples. A syringe rinsed with stock heparin solution contained a sufficient amount of heparin to prevent coagulation of blood drawn in the syringe.

C. Methods

1. Virus preparations:

a) Challenge strain virus: The contents of a vial of seed virus received from the Vineland Poultry Laboratory was dissolved in 1 ml of the diluent. The solution, so obtained, was allowed to stand for half an hour. The supernatant was removed and mixed in equal parts with fresh diluent and kept at room temperature for an hour. Then the virus was inoculated onto CAM's of 12 day old embryonated eggs and, following 5 days incubation at 38°C., the CAM's with confluent growth were collected. The CAM's were minced with scissors and ground with glass beads in a mortar. A sufficient quantity of N-Z-Amine was added to give 10% suspension of the stock virus. Aliquots of the suspension stored at -20°C. and one sample representing the group of vials were titrated on CAM's to determine the titer of the stock virus preparation.

b) Vaccine strain virus: Originally, the Va virus was obtained from the Vineland Laboratories as a lyophilized virus suspension. The stock Va virus was prepared in the same manner as was the Ch virus. The stock virus was maintained on CAM's of embryonated eggs. The suspension of CAM was stored in screw capped

vials in the frozen state. Before the experimental infection of birds with the stock Va virus it was passaged on CAM's and a fresh 10% suspension of CAM was prepared and stored in aliquots in the frozen state.

c) Outbreak strain virus: Ou virus was obtained as a suspension of CAM's. The suspension was immediately passed on CAM after being received from New Jersey State Poultry Diagnostic Laboratory and a 10% suspension of CAM was prepared and stored in vials in the frozen state.

2. Egg inoculation: Prior to inoculation, eggs were incubated for 12 days at 38°C. with 80 percent relative humidity in an egg incubator (Jamesway, model 252, Ft. Atkinson, Wisconsin). Each egg was candled and an area including the air sac and a well developed area of the CAM which was free from prominent blood vessels was marked. The air sac end of the egg was swabbed with iodine solution and a small hole was drilled with a sterile needle over the center of the air space. Then the eggs were placed in a horizontal position and were swabbed with iodine solution on the marked area on the shell and allowed to dry. On the marked area two lines crossing each other at a 90° angle were drilled with a round dental burr, deep enough to loosen a flap of shell, but not enough to penetrate the shell membrane. If profuse bleeding occurred as a result of injury of

the shell membrane, the eggs were discarded. The area of shell around the hole was gently swabbed with a sterile cotton swab soaked in absolute alcohol. After disinfecting the shell membrane, a drop of sterile diluent was placed on the exposed shell membrane and allowed to remain there for at least one minute to moisten the shell membrane. The moistened shell membrane was then punctured with an inoculating needle. A twenty-one gauge needle was placed on the shell membrane cut surface down and was pushed as gently as possible to make a cut on the shell membrane. The CAM was dropped by gentle suction with a rubber teat, thus creating an artificial air space along the axis of the egg. Suction was applied over the opening previously drilled in the air space. Bleeding indicated injury of the CAM or its blood vessels and these eggs were discarded.

The inoculum (0.1 to 0.25 ml prepared material) was introduced into the CAM from a disposable tuberculin syringe fitted with a #26 gauge 3/8 inch needle. The inoculated eggs were rolled gently to distribute the inoculum evenly on the dropped area of CAM. Both openings on the inoculated eggs were closed with transparent cellulose tape and the eggs were then placed on their long axis in trays with the artificial air space uppermost and incubated undisturbed at 38°C. for 5 days.

3. Experimental infection of birds: Groups of birds were experimentally infected by intradermal, intravenous and intratracheal routes with (Ch) virus and by intradermal and intravenous routes with (Va) virus. Some birds were infected intradermally with (Ou) virus. For immunity tests previously infected birds were challenged intradermally with (Ch) or (Ou) virus.

a) Intradermal injections: Five to six week old birds were used for intradermal inoculations. The combs of the birds cleaned by swabbing with distilled water and using an ordinary cork fitted with 8-10 dressmakers pins. One surface of the comb was scarified to break the continuity of the skin. Every possible care was taken not to cause hemorrhage during the scarification. About 0.04 to 0.05 ml of undiluted stock virus preparation was rubbed on the scarified face of the comb. control birds were scarified in the same manner and the same amount of sterile diluent was rubbed on. In some birds the other side of the comb was also inoculated with sterile diluent after scarification.

b) Intravenous injections: Cockerels and hens were injected intravenously with 0.5 ml of undiluted stock virus preparation. The left wing vein was used for inoculation. Control birds received the same amount of sterile diluent by the same route.

c) Intratracheal injections: Groups of birds about 4 weeks old were used for intratracheal inoculations, 0.05 to 0.1 ml of undiluted stock virus preparation was injected directly into the trachea from a tuberculin syringe fitted to a #27 gauge needle.

4. Collection of samples: Birds infected by the intravenous route were killed at intervals after inoculation. A bird was killed right after the injection as a 0 time sample and then another at 15 minutes, 30 minutes and 1 hour. Birds were killed every hour, thereafter, for the next 24 hours. Daily samples were then obtained by killing a bird every day for 25-30 days after inoculation. Birds were bled to death from the heart. To bleed from the heart the chicken was laid on its side on a table and the wings and legs were held by an assistant or tightened with tape. A 30 ml syringe fitted to an 18 gauge, 2 inch long needle was used for bleeding. The syringe was moistened with heparin solution. The point of fusion of the two clavicles, where they form the V-shaped furcure, was used as a guide in locating the depression immediately dorsal to that point. The needle was inserted vertically dorsal to the depression. A slight negative pressure was maintained in the syringe by slowly withdrawing the plunger. The needle was advanced until blood flowed into the syringe. The needle was then maintained in position by holding it with one hand and the

plunger was slowly withdrawn with the other hand. Birds were exanguinated as completely as possible at the time they were killed.

a) Blood samples: Portions of the blood were transferred into 100 x 8 mm sterile test tubes and centrifuged at 1000 rpm for 15 minutes. A sample of plasma was taken and the rest of the plasma was pipetted off. The buffy coat of leucocytes in each tube was then removed carefully with a tuberculin syringe and transferred into vials containing a measured amount of sterile diluent to give approximately a 1:10 dilution of buffy coat. Red blood cells were withdrawn by pipette from the bottom of the tube and mixed with sterile diluent to make a 1:5 dilution. The vials containing undiluted plasma, 1:10 dilution of buffy coat and 1:5 dilution of red blood cells were labeled and stored at -20°C . until they were titrated.

b) Comb, wattle and skin samples: The comb and wattles of each bird were removed and placed in a sterile petri dish and labelled. Skin samples were always taken from the area where pock lesions appeared on the chest and from another area where no visible pocks were seen. A skin sample about 2 cm square was excised with a pair of scissors for each skin sample.

c) Collection of tissue specimens: All birds sacrificed for sample collection were autopsied and specimens of organs were excised

for viral assay. Tissue samples consisting of whole organs or small portions of organs were obtained by aseptic techniques. Whole spleen, brain and lungs; one half of the kidney and a piece of liver were removed using sterile instruments for each sample collected. Specimens were put in sterile petri dishes and stored at -20°C . until the inoculating material was prepared.

5. Preparation of samples for virus assay: To prevent possible viral contamination of individual specimens by blood or other fluids, all samples were rinsed individually in sterile N-Z-Amine solution. Each specimen was then weighed and ground thoroughly in a mortar containing sterile glass beads. The appropriate volume of N-Z-Amine with antibiotics was added to make a 20% solution. After standing 20-30 minutes at room temperature the suspension was centrifuged at 2,000 rpm for 20 minutes. The supernatant was withdrawn and stored in screw-capped small vials in 2-3 ml quantities in the frozen state.

6. Virus assay and determination of titer: Suspensions of samples were thawed and inoculated on the CAM of embryonated eggs. Eggs were incubated 12 days before inoculation. Suspensions that produced viral pocks in numbers too great to count were diluted in N-Z-Amine and re-assayed. One tenth of each tissue suspension or respective dilution and

0.1 to 0.25 ml of blood samples were inoculated into 3-4 eggs and incubated at 38°C. for five days. At the end of 5 days incubation the eggs were chilled in a refrigerator for a couple of hours before opening. The egg shell on the artificial air space was disinfected with iodine solution and an opening was cut at the artificial air space with the help of a pair of scissors. The CAM was then removed and floated in a petri dish containing diluent. The pocks were counted with the aid of an illuminator if necessary. The number of virus particles per ml or grams of assayed material was determined by averaging the number of pocks per membrane and multiplying by 10 for conversion to ml or gram basis, and by the dilution factor. If 0.25 ml blood samples were injected, the average pock count from those blood samples was multiplied by 4 for conversion to ml basis.

IV. EXPERIMENTAL RESULTS

A. Experiment I. Intradermal inoculations (ID)

1. Clinical features of fowlpox in intradermally infected birds: The outstanding clinical features of fowlpox in birds infected intradermally were the primary pock lesions which formed on the scarified areas of the combs of the chickens. On the chickens combs, infected with challenge strain virus, there was no pathological changes for the first 3 days after inoculation. By the 4th day, small yellowish areas developed at the site of injury. These lesions were few in number and measured less than 1 mm in diameter. By the 5th or 6th day the lesions were numerous and the yellowish areas became white, slightly raised papules which increased in size until they were about 1 to 2 mm diameters. Because of the closely spaced injuries on the combs and the large amount of virus inoculated, the lesions at the vesicular stage were so numerous and aggregated as to become confluent, forming thick, extensive lesions on the reddened and slightly bleeding comb. The lesions underwent a process of desquamation and scab formation during the second week after inoculation (Figure 1). Detachment of the crusts and healing of the lesions took place during the third week past inoculation. Eventually, the large, brown, hard, shrunken crusts dropped off leaving a smooth scar.

Figure 1. Primary lesions on the comb of chicken 14 days after intradermal inoculation with the challenge strain of fowl poxvirus.

Although the lesions were seen on the 3rd day after inoculation of vaccine strain virus in the particular bird shown in Figure 2, generally lesions were visible on the 4th or 5th days after inoculation of vaccine strain virus. Except that there was a shorter time for the development of new epithelium over lesions produced by the vaccine strain than by the challenge or outbreak strains of virus, strains of virus had little effect on the appearance of primary lesions.

2. Growth curve of virus in primary lesions after ID infection: The curves showing the virus growth in relation to visible primary lesions on combs are summarized in Figures 2 and 3. Very small quantities of virus were found in the samples taken 3 to 4 minutes after the inoculation (Tables 20 and 21). Virus was not detected on the 1st day post inoculation and first reappeared on the 2nd day. The titer of challenge strain virus was 6×10^2 PFU/gm when first detected in the comb (Table 20). The titer increased rapidly on the 3rd, 4th and 5th days and reached a maximum of 9×10^7 PFU/gm on the 9th day. The titer remained stationary at about the same high level until the 11th day and then fell steadily from the 12th day onwards until virus was not detectable on the 27th day. The persistence of challenge strain virus was related to the development of the clinical features of the disease, being present in greatest amount when the lesions were fully developed on the infected combs.

Figure 2. Growth curve of challenge strain virus in the primary lesions of chickens inoculated on the comb with 0.05 ml of virus (3×10^7 PFU)

Figure 3. Growth curve of vaccine strain virus in the primary lesions of chickens inoculated on the comb with 0.05 ml of virus (2×10^6 PFU)

TABLE 20

RELATION BETWEEN THE TITER OF VIRUS IN PRIMARY LESIONS ON COMBS AND THE CLINICAL FEATURES OF THE DISEASE IN CHICKENS INFECTED INTRADERMALLY WITH CHALLENGE STRAIN VIRUS (3×10 PFU)

Birds Examined On Day After Infection	No.	Titer of Virus*	Clinical Feature
0	2	5×10^3	No lesion
1	1	N	"
2	1	6×10^2	"
3	2	7×10^4	"
4	1	3×10^5	Small lesions
5	2	8×10^6	Papules
6	1	2×10^7	"
7	1	2×10^7	Vesicles
8	1	9×10^7	Confluent lesion
9	2	3×10^7	"
10	1	5×10^7	"
11	2	8×10^6	"
12	1	7×10^5	Desiccation and crust formation
13	1	4×10^5	"
14	2	6×10^5	"
15	1	3×10^6	"
16	1	6×10^5	"
18	1	3×10^4	"
20	1	6×10^4	"
22	1	N	Crust dropped off
23	1	9×10^2	Small crust
25	1	2×10^2	"
29	1	N	Crust dropped off
33	1	N	New epithelium formed

* Virus titres in combs in PFU/gm

N = Negative for Virus

TABLE 21

RELATION BETWEEN THE TITER OF VIRUS IN PRIMARY LESIONS ON COMBS AND THE CLINICAL FEATURES OF THE DISEASE IN CHICKENS INFECTED INTRADERMALLY WITH VACCINE STRAIN VIRUS (2 x 10 PFU)

Birds Examined On Day After Infection	No.	Titer of Virus*	Clinical Feature
0	1	5 x 10 ²	No lesion
1	2	N	"
2	1	3 x 10 ⁴	"
3	2	5 x 10 ⁵	Small lesions
4	1	6 x 10 ⁶	Papules
5	2	3 x 10 ⁶	"
6	2	3 x 10 ⁶	Vesicles
7	1	4 x 10 ⁶	Confluent lesion
8	2	7 x 10 ⁶	"
9	1	7 x 10 ⁴	Desiccation
10	1	7 x 10 ⁴	Crust formation
11	2	6 x 10 ⁴	"
12	1	7 x 10 ³	A large dried crust
13	1	8 x 10 ²	"
14	1	N	Crust dropped off
15	1	N	New epithelium
16	1	N	"
17	1	N	"
18	1	N	"
19	1	N	"
20	1	N	"
21	1	N	"
22	1	N	"

* Virus titers in combs in PFU/gm

N = Negative for Virus

The titer of the vaccine strain virus was 3×10^2 PFU/gm on first detection in the comb on the 2nd day post inoculation (Table 21).

The titer of virus increased on the 3rd day and reached a maximum of 6×10^6 PFU/gm on the 4th day. The titer of vaccine strain virus remained at about the same level until the 8th day, then fell steadily until it was not detected in combs on the 14th day post inoculation.

Although there was a difference between the amount of challenge and vaccine strain virus on first detection on the 2nd day, the curves of virus in primary lesions produced by these strains were essentially the same, but the duration of persistence of virus was shorter and the virus concentration was somewhat lower with vaccine strain virus than challenge strain virus.

The general condition of the chickens infected intradermally remained normal. While the inoculations induced typical primary lesions, more or less extensive depending on the extent of injury by scarification, no secondary lesions developed. Birds which were not killed for sample collection showed immunity when they were challenged intradermally or intravenously two months after the experimental inoculations with outbreak or challenge strain virus.

3. Virus in blood and internal organs after ID infection: There was little change from normal observed by macroscopic examination of internal

organs of infected birds. Weights of internal organs of chickens infected intradermally were generally the same as the weights of internal organs of chickens infected intratracheally and those killed within 24 hours after intravenous inoculation (Tables 1, 2, 3, 4 and 7).

Virus was not found in blood of birds infected intradermally with either challenge or vaccine strain virus. Internal organs were usually found to be free of virus (Table 9) except that the lungs of two birds infected with challenge strain virus did contain some virus (Table 8). The lungs of the birds killed on the 7th day had 20 PFU/gm in one of the three eggs inoculated. The result was not confirmed with the rest of the sample. The lung from the bird killed on the 10th day contained 800 PFU/gm.

In a separate experiment three birds were infected intradermally with challenge strain virus on extensively scarified combs. Of the three birds, one showed a low titer of virus in the buffy coat fraction of the blood. Virus was not detected in the internal organs of any of the three birds killed 24 hours post inoculation.

B. Experiment II. Intravenous inoculations (IV)

1. Disappearance of virus from circulation: Two sets of experiments were conducted to study the disappearance of fowlpox virus from the circulation following intravenous inoculation of the challenge and vaccine

TABLE 1
WEIGHTS (IN GRAMS) OF INTERNAL ORGANS OF BIRDS INFECTED
INTRADERMALLY WITH THE CHALLENGE
STRAIN OF FOWLPOX VIRUS

Age of Birds	Days After Infection	Spleen	Kidney	Liver	Lung
30 days	0	0.6	2.8	11.4	2.8
31	1	0.6	2.8	11.3	3.4
32	2	0.7	3.2	12.0	3.2
33	3	0.7	3.2	12.4	3.6
34	4	0.8	3.7	13.6	3.2
35	5	0.6	4.0	13.0	2.8
36	6	0.7	3.9	11.2	3.0
37	7	0.9	4.0	14.8	3.0
38	8	0.7	3.6	14.0	3.2
39	9	1.15	4.2	14.8	2.8
40	10	0.6	3.7	13.6	3.6
41	11	0.8	5.0	14.4	3.4
42	12	0.9	5.2	14.8	3.9
43	13	0.6	3.35	15.2	3.2
44	14	0.8	4.0	19.2	3.8
45	15	1.2	4.8	16.6	4.2
46	16	1.1	5.0	18.5	5.0
48	18	1.2	4.8	19.2	4.4
50	20	1.3	4.6	24.0	3.6
52	22	1.8	5.4	18.7	4.2
53	23	1.4	5.3	22.4	4.6
55	25	1.6	7.1	27.0	5.2
59	29	1.8	NW	NW	NW
63	33	1.7	NW	NW	NW

TABLE 2
WEIGHTS (IN GRAMS) OF INTERNAL ORGANS OF BIRDS INFECTED
INTRADERMALLY WITH THE VACCINE
STRAIN OF FOWLPOX VIRUS

Age of Birds	Days After Infection	Spleen	Kidney	Liver	Lung
28 days	0	0.5	2.8	11.2	2.8
29	1	0.4	2.8	11.0	4.0
30	2	0.5	2.6	11.5	3.2
31	3	0.6	2.7	12.0	2.7
32	4	0.9	2.8	11.2	2.6
33	5	0.6	3.0	11.5	2.4
34	6	0.6	2.5	10.0	3.0
35	7	0.6	3.5	11.5	2.4
36	8	1.0	2.5	14.6	2.8
37	9	1.0	3.7	13.0	3.6
38	10	0.9	4.5	14.8	3.6
39	11	0.65	5.2	17.5	3.6
40	12	0.9	4.0	13.3	3.8
41	13	0.9	4.2	19.6	3.8
42	14	0.8	3.5	18.0	4.0
43	15	0.8	4.2	17.0	3.6
44	16	0.9	4.2	19.0	3.6
45	17	0.9	3.6	17.0	3.4
46	18	0.9	4.6	21.0	3.7
47	19	2.0	5.2	26.0	3.6
48	20	1.0	4.4	18.6	5.2
49	21	1.1	4.6	23.8	4.7
50	22	1.4	5.4	24.0	4.7

TABLE 3
WEIGHTS (IN GRAMS) OF INTERNAL ORGANS OF BIRDS INFECTED
INTRAVENOUSLY WITH THE CHALLENGE
STRAIN OF FOWLPOX VIRUS

Age of Birds	Time After Infection	Spleen	Kidney	Liver	Lung
30 days	0 Time	0.6	2.8	10.2	2.0
30	15 Min.	0.6	2.8	9.2	2.1
30	30	0.7	3.2	12.0	2.3
30	1 Hour	0.7	3.2	11.4	2.1
30	2	0.8	3.1	13.6	2.3
30	3	0.6	3.0	9.6	1.9
30	4	0.7	3.9	12.2	2.2
31	5	0.8	3.9	13.8	2.3
31	6	0.7	3.6	10.0	1.8
31	7	0.8	3.2	14.5	4.2
31	8	0.6	3.6	12.8	2.3
31	9	0.7	3.2	13.4	1.9
31	10	0.8	3.0	9.8	3.0
33	11	0.9	4.2	11.6	2.2
33	12	0.6	4.3	13.4	2.6
33	13	1.1	3.8	12.4	2.6
33	14	0.6	2.6	14.0	3.2
33	15	0.5	3.4	9.6	2.8
33	16	0.7	2.8	13.2	3.4
33	17	1.1	4.0	14.5	2.5
33	19	0.8	4.2	10.9	3.8
33	21	0.9	3.8	13.4	2.9
33	23	0.6	2.6	12.4	3.0

TABLE 4
WEIGHTS (IN GRAMS) OF INTERNAL ORGANS OF BIRDS INFECTED
INTRAVENOUSLY WITH THE VACCINE
STRAIN OF FOWLPOX VIRUS

Age of Birds	Time After Injection	Spleen	Kidney	Liver	Lung
30 days	0 Time	1.0	3.2	13.4	3.0
30	15 Min.	0.5	2.4	10.5	2.0
30	30	0.6	3.2	10.8	2.2
30	1 Hour	0.6	3.2	13.6	2.5
30	2	0.5	2.4	10.8	2.8
30	3	0.4	3.2	10.5	1.6
30	4	0.5	3.6	13.0	1.8
31	5	0.6	4.2	11.4	2.6
31	6	0.7	4.0	9.5	2.8
31	7	0.6	3.2	11.8	3.0
31	8	0.8	3.0	15.7	2.8
31	9	0.6	4.0	12.6	3.2
31	10	0.8	3.2	10.4	3.4
32	11	1.0	3.6	11.3	3.0
32	12	1.1	3.4	11.2	2.6
32	13	0.8	3.8	14.8	3.8
32	14	1.1	4.0	12.6	2.2
32	15	1.2	4.2	13.2	2.4
32	16	1.1	3.6	11.5	2.5
32	17	0.9	3.8	14.7	2.7
32	19	0.8	3.4	11.8	3.0
32	21	0.7	2.8	10.4	3.6
32	23	0.9	3.0	12.6	3.1

TABLE 7

WEIGHTS (IN GRAMS) OF INTERNAL ORGANS OF BIRDS INFECTED
INTRATRACHEALLY WITH THE CHALLENGE
STRAIN OF FOWLPOX VIRUS

Age of Birds	Days After Injection	Spleen	Kidney	Liver	Lung
28 days	0 time	0.6	2.8	9.4	2.2
29	1 day	0.9	3.2	13.4	2.6
29	1	0.7	2.6	12.0	2.3
30	2	0.9	3.0	11.5	1.8
30	2	0.8	2.8	13.0	2.1
31	3	1.1	3.4	14.5	2.6
31	3	0.9	2.9	12.0	2.3
32	4	1.2	3.4	14.0	2.8
32	4	0.6	3.2	10.6	2.1
33	5	0.9	3.2	13.6	2.4
33	5	1.2	3.0	15.0	2.8
34	6	1.1	3.2	19.5	3.6
34	6	0.7	2.7	13.6	2.4
35	7	1.3	3.2	15.0	3.0
35	7	0.8	2.4	13.0	2.6
36	8	1.3	3.4	16.5	3.9
36	8	1.4	3.0	17.0	4.2
37	9	0.7	2.4	13.5	2.6
37	9	1.1	2.6	12.5	2.8
38	10	1.3	3.2	14.6	4.2
39	11	0.9	3.0	13.5	2.7
40	12	0.9	2.6	16.5	3.3
41	13	1.3	3.4	17.0	3.6
42	14	1.1	3.9	13.8	2.8
43	15	1.4	3.3	17.0	3.6
44	16	1.2	3.2	14.4	3.2

TABLE 8
 PRESENCE OF VIRUS IN BLOOD AND VARIOUS ORGAN
 SAMPLES OF BIRDS INFECTED INTRADERMALLY WITH
 CHALLENGE STRAIN OF FOWLPOX VIRUS

Days After Injec.	Comb	Blood			Spleen	Kidney	Liver	Lung	Brain
		Pla.	RBC	Coat					
0	P	N	N	N	N	N	N	N	N
1	N	N	N	N*	N	N	N	N	N
2	P	N	N	N	N	N	N	N	N
3	P	N	N	N	N	N	N	N	N
4	P	N	N	N	N	N	N	N	N
5	P	N	N	N	N	N	N	N	N
6	P	N	N	N	N	N	N	N	N
7	P	N	N	N	N	N	N	P**	N
8	P	N	N	N	N	N	N	N	N
9	P	N	N	N	N	N	N	N	N
10	P	N	N	N	N	N	N	P***	N
11	P	N	N	N	N	N	N	N	N
12	P	N	N	N	N	N	N	N	N
13	P	N	N	N	N	N	N	N	N
14	P	N	N	N	N	N	N	N	N
15	P	N	N	N	N	N	N	N	N
16	P	N	N	N	N	N	N	N	N
18	P	N	N	N	N	N	N	N	N
20	P	N	N	N	N	N	N	N	N
22	P	N	N	N	N	N	N	N	N
23	P	N	N	N	N	N	N	N	N
25	P	N	N	N	N	N	N	N	N
29	N	N	N	N	N	N	N	N	N
33	N	N	N	N	N	N	N	N	N

P = Positive for Virus

N = Negative for Virus

* = In separate experiment virus was found in one of the three birds tested.

** = 20 PFU/gm of virus were found in one egg only.

*** = 800 PFU/gm of virus were found.

TABLE 9
 PRESENCE OF VIRUS IN BLOOD AND VARIOUS ORGAN
 SAMPLES OF BIRDS INFECTED INTRADERMALLY WITH
 VACCINE STRAIN OF FOWLPOX VIRUS

Days After Injec.	Comb	Blood			Spleen	Kidney	Liver	Lung	Brain
		Pla.	RBC	Buffy Coat					
0	P	N	N	N	N	N	N	N	N
1	N	N	N	N	N	N	N	N	N
2	P	N	N	N	N	N	N	N	N
3	P	N	N	N	N	N	N	N	N
4	P	N	N	N	N	N	N	N	N
5	P	N	N	N	N	N	N	N	N
6	P	N	N	N	N	N	N	N	N
7	P	N	N	N	N	N	N	N	N
8	P	N	N	N	N	N	N	N	N
9	P	N	N	N	N	N	N	N	N
10	P	N	N	N	N	N	N	N	N
11	P	N	N	N	N	N	N	N	N
12	P	N	N	N	N	N	N	N	N
13	P	N	N	N	N	N	N	N	N
14	N	N	N	N	N	N	N	N	N
15	N	N	N	N	N	N	N	N	N
16	N	N	N	N	N	N	N	N	N
17	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	N	N
19	N	N	N	N	N	N	N	N	N
21	N	N	N	N	N	N	N	N	N
22	N	N	N	N	N	N	N	N	N

P = Positive for Virus
 N = Negative for Virus

strains of fowlpox virus. Each bird was injected intravenously with approximately 3×10^8 PFU of challenge strain or 2×10^7 PFU of vaccine strain virus. The first samples from each group were collected right after the injection of the virus into the wing vein. Blood drawn from the heart was centrifuged to separate the plasma from the particulate components of the blood. Following the injection of 3×10^8 PFU of challenge strain virus into the vein, if the average blood volume of the birds in this experiment was 50 ml (Ball, 1965), each ml of blood should have contained 6×10^6 PFU due to dilution. Of the 6×10^6 PFU/ml of challenge strain virus only 2×10^4 PFU/ml were detected in the buffy coat and 2×10^3 PFU/ml in the RBC fraction in the first sample taken 3-4 minutes after injection (Table 15). Similar results were obtained in the experiment with the vaccine strain of fowlpox virus. Of the 4×10^5 PFU/ml of vaccine strain virus injected 9×10^3 PFU/ml were detected in the buffy coat and 3×10^3 PFU/ml in the RBC fraction in the first sample (Table 16). In the early samples only small quantities of virus, not more than 80 PFU/ml were found in the plasma. Although virus was detected in plasma collected as late as three hours after injection of virus the very low titers of the virus in the plasma indicated that the virus was primarily associated with the particulate components of the blood.

TABLE 15

DISAPPEARANCE OF THE CHALLENGE STRAIN VIRUS FROM THE CIRCULATION AND
ITS ACCUMULATION IN INTERNAL ORGANS OF CHICKENS INOCULATED
INTRAVENOUSLY WITH 0.5 ml OF VIRUS (3×10 PFU)

Hours After Infection	Blood			Titer of Virus			
	Pla.	RBC	Buffy Coat	Spleen	Kidney	Liver	Lung
0	80	2×10^3	2×10^4	7×10^2	6×10^2	5×10^2	9×10^3
15 Min.	20	7×10^3	8×10^3	7×10^3	8×10^2	6×10^2	6×10^3
30	10	6×10^3	2×10^2	8×10^3	4×10^2	4×10^2	6×10^2
1 Hour	30	8×10^3	2×10^2	8×10^3	2×10^2	4×10^1	3×10^3
2	10	7×10^2	6×10^3	9×10^3	1×10^2	1.6×10^1	2×10^2
3	20	6×10^3	2×10^3	8×10^4	3×10^1	4×10^1	2×10^3
4	N	4×10^2	6×10^3	8×10^4	5×10^2	4×10^2	8×10^2
5	N	3×10^2	5×10^3	9×10^3	5×10^1	5×10^1	4×10^2
6	N	4×10^1	7×10^3	8×10^3	2×10^1	4×10^1	1×10^2
7	N	3×10^2	6×10^3	9×10^4	N	N	3×10^2
8	N	N	2×10^2	2×10^2	6×10^1	2×10^1	2×10^2
9	N	N	8×10^2	3×10^2	N	N	1×10^2
10	N	N	3×10^2	3×10^2	N	N	2×10^2
11	N	N	2×10^2	4×10^2	N	N	1.5×10^2
12	N	N	N	5×10^2	N	N	2×10^2
13	N	N	2×10^2	1×10^2	N	N	4×10^2
14	N	N	N	N	N	N	3×10^2
15	N	N	N	N	N	N	5×10^2
16	N	N	N	N	N	N	5×10^2
17	N	N	N	N	N	N	2×10^2
19	N	N	N	N	N	N	1.5×10^2
21	N	N	N	N	N	N	1×10^2
23	N	N	N	N	N	N	8×10^2

TABLE 16

DISAPPEARANCE OF THE VACCINE STRAIN VIRUS FROM THE CIRCULATION AND
ITS ACCUMULATION IN INTERNAL ORGANS OF CHICKENS INOCULATED
INTRAVENOUSLY WITH 0.51 ml of VIRUS (2×10 PFU)

Hours After Infection	Blood			Spleen	Titer of Virus		Lung
	Pla.	RBC	Buffy Coat		Kidney	Liver	
0	20	3×10^3	9×10^3	6.5×10^2	7×10^2	9×10^2	9×10^2
15 Min.	20	4×10^3	8×10^3	6×10^3	9×10^2	6×10^2	2×10^3
30	10	7×10^2	9×10^3	9×10^3	6×10^2	5×10^2	9×10^2
1 Hour	20	2×10^2	4×10^3	8×10^4	3×10^2	8×10^2	6×10^2
2	10	1×10^2	2×10^3	9×10^4	1.5×10^2	3×10^2	5×10^2
3	30	1×10^2	3×10^3	9×10^4	9×10^1	6×10^1	7×10^2
4	N	1.2×10^2	9×10^2	8×10^4	5×10^2	8×10^1	9×10^2
5	N	7.5×10^1	8×10^2	8×10^4	8×10^1	4×10^1	8×10^1
6	N	1.2×10^2	6×10^2	6×10^3	9×10^1	N	5×10^2
7	N	N	6×10^2	2×10^2	8×10^1	N	5×10^2
8	N	N	2×10^2	3×10^2	5×10^1	N	8×10^1
9	N	N	3×10^2	3×10^2	N	N	4×10^2
10	N	5×10^1	8×10^1	2×10^2	N	N	2×10^2
11	N	N	3×10^2	N	N	N	N
12	N	N	8×10^1	3×10^2	4×10^1	N	8×10^1
13	N	N	3×10^2	4×10^2	N	N	N
14	N	N	N	N	N	N	N
15	N	N	N	N	N	N	N
16	N	N	N	N	N	N	N
17	N	N	N	N	N	N	N
19	N	N	N	N	N	N	N
21	N	N	N	N	N	N	N
23	N	N	N	N	N	N	N

The disappearance of virus from circulation was rather rapid. By six hours post-injection virus was no longer detected in the RBC fraction, although the buffy coat fraction still contained small quantities of virus in samples collected up to the 15th hour. Virus was not detected in any fraction of blood collected after the 15th hour (Tables 10 and 11). The titers of virus in the RBC fraction and the buffy coat fraction were about the same up to 30 minutes post-injection, but in samples taken 1 hour post-injection more virus was detected in the buffy coat fraction than in the RBC fraction. In Figures 4 and 5 the results of the experiments with challenge strain virus and vaccine strain virus just discussed are presented graphically.

2. Accumulation and disappearance of virus in internal organs: The results of titrations for virus content of various organs which were collected at hourly intervals after the virus injection are shown (Tables 10 and 11). The collection and preparation of samples from which these figures were derived are described in the section on materials and methods. The results obtained from the experiments with challenge strain and vaccine strain viruses were essentially similar (Figures 4 and 5). The titer of virus in kidney and liver was not different in experiments with challenge strain virus and vaccine strain virus. Virus persisted in these organs in small quantities until the 8th hour after inoculation. The accumulation of

TABLE 10

PRESENCE OF VIRUS IN BLOOD AND VARIOUS ORGAN
SAMPLES OF BIRDS INFECTED INTRAVENOUSLY WITH
THE CHALLENGE STRAIN OF FOWLPOX VIRUS

Hours After Injection	Plasma	Blood RBC	Buffy Coat	Spleen	Kidney	Liver	Lung
0*	P	P	P	P	P	P	P
15 Min.	P	P	P	P	P	P	P
30	P	P	P	P	P	P	P
1 Hour	P	P	P	P	P	P	P
2	P	P	P	P	P	P	P
3	P	P	P	P	P	P	P
4	N	P	P	P	P	P	P
5	N	P	P	P	P	P	P
6	N	P	P	P	P	P	P
7	N	P	P	P	N	N	P
8	N	N	P	P	P	P	P
9	N	N	P	P	N	N	P
10	N	N	P	P	N	N	P
11	N	N	P	P	N	N	P
12	N	N	N	P	N	N	P
13	N	N	P	P	N	N	P
14	N	N	N	N	N	N	P
15	N	N	N	N	N	N	P
16	N	N	N	N	N	N	P
17	N	N	N	N	N	N	P
19	N	N	N	N	N	N	P
21	N	N	N	N	N	N	P
23	N	N	N	N	N	N	P

P = Positive for Virus

N = Negative for Virus

* = 3-4 Minutes after injection

TABLE 11
 PRESENCE OF VIRUS IN BLOOD AND VARIOUS ORGAN
 SAMPLES OF BIRDS INTRAVENOUSLY INFECTED WITH
 THE VACCINE STRAIN OF FOWLPOX VIRUS

Hours After Injection	Plasma	Blood RBC	Buffy Coat	Spleen	Kidney	Liver	Lung
0*	P	P	P	P	P	P	P
15 Min.	P	P	P	P	P	P	P
30	P	P	P	P	P	P	P
1 Hour	P	P	P	P	P	P	P
2	P	P	P	P	P	P	P
3	P	P	P	P	P	P	P
4	N	P	P	P	P	P	P
5	N	P	P	P	P	P	P
6	N	P	P	P	P	N	P
7	N	N	P	P	P	N	P
8	N	N	P	P	P	N	P
9	N	N	P	P	N	N	P
10	N	P	P	P	N	N	P
11	N	N	P	N	N	N	N
12	N	N	P	P	P	N	P
13	N	N	P	P	N	N	N
14	N	N	N	N	N	N	N
15	N	N	N	N	N	N	N
16	N	N	N	N	N	N	N
17	N	N	N	N	N	N	N
19	N	N	N	N	N	N	N
21	N	N	N	N	N	N	N
23	N	N	N	N	N	N	N

P = Positive for Virus

N = Negative for Virus

* = 3-4 minutes after injection

Figure 4. Disappearance of challenge strain virus from circulation and its accumulation in internal organs of chickens inoculated intravenously with 0.5 ml of virus (3×10^8 PFU)

Figure 5. Disappearance of vaccine strain virus from the circulation and its accumulation in internal organs of chickens inoculated intravenously with 0.5 ml of virus (2×10^7 PFU)

challenge strain virus in the spleen increased with time, reaching a maximum titer of 8×10^4 PFU/ml on 3rd and 4th hour post-injection. Virus persisted in the buffy coat fraction of the blood and in the spleen until 14 hours post-inoculation. The titer in the spleen was somewhat higher than the titer of virus in the buffy coat fraction.

The lung was the only organ in which challenge strain virus was detected in all samples collected at hourly intervals for the entire 24 hours. Virus disappeared from the lungs of chickens injected with vaccine strain virus at 12 hours post-injection. In the birds injected with the challenge strain virus all samples had about the same amount of virus. The challenge strain virus was present in almost the same titer in samples taken at 1 day and 2 days post-inoculation, also.

3. Clinical features of fowlpox in birds infected intravenously: The characteristic clinical feature of the disease in the intravenously infected chickens in this experiment was the development of secondary lesions. All the chickens infected intravenously with either strain of virus developed secondary lesions. The pox lesions were usually present on both sides of the comb and on the wattles. Typical pox lesions also developed on the eyelids, beak and skin of the infected birds. The lesions developed on the feet were somewhat different than the lesions seen on the comb or skin.

The flat and small lesions on the feet became dried in a short time and only dark centered brown areas remained 4 or 5 days after the appearance of the lesions.

The disease which developed after intravenous inoculation was more severe than that following intradermal or intratracheal inoculation. Five birds died in the two experiments and many of the chickens were sacrificed for sample collection at the height of the disease. Some birds did not develop severe disease and they recovered. The general condition of the chickens was normal until the 11th day. Loss of weight was obvious in most of the chickens by the 12th day post-injection. The birds were debilitated and they sat in one place with a dejected aspect. The birds which suffered from the severe disease did not resist when they were handled. There was general advanced emaciation. The lesions about the eyelids were often very extensive, involving the margins of the lids, partially or completely closing the aperture.

The following description of the lesions applies to chickens which were inoculated with the challenge strain of virus and which did not develop the severe disease. Individual lesions were first seen on the 5th day as a few small pinpoint areas on the wattles (Figure 6). The number of these lesions increased for two days finally covering the surface of the

Figure 6. First appearance of lesions on the wattles and comb of a chicken 5 days after intravenous inoculation with the challenge strain of fowl poxvirus.

wattles and comb. Within the next 2 days these areas developed white papules which increased in size attaining 2 to 3 mm in diameter. By the 9th day the papules developed into slightly raised vesicles as they increased in size and the color of lesions changed to yellow and then to brown. At this stage of the disease lesions were apparent on the eyelids, beak and on the skin (Figures 7 and 8). Lesions developed on the feet also (Figure 9). The lesions on the comb and wattles were moist for a short time but soon dried and showed areas of inflammation at the base. Crusting began by the third week, forming the dark brown or black nodules which attained a size of about 4 to 5 mm in diameter. After the crust dropped off a smooth scar developed, although in mild cases there was no evidence of scar tissue. The clinical features of the disease were almost the same in chickens inoculated intravenously with the vaccine strain of fowl poxvirus.

4. Growth curve of virus in secondary lesions after IV injections: The pattern of the growth curve of virus in the secondary lesions on the comb corresponded to the pattern of the growth curve of virus in primary lesions. The titers of virus in daily samples of combs in relation to the clinical features of the disease are shown in Tables 22 and 23. Virus was not detected in combs until 2 days after inoculation. In birds inoculated with

Figure 7. Secondary lesions on the head of a chicken 13 days after intravenous inoculation with the challenge strain of fowl poxvirus.

Figure 8. Secondary lesions on the head of a chicken 15 days after intravenous inoculation with the challenge strain of fowl poxvirus.

Figure 9. Secondary lesions on the legs of a chicken 13 days after intravenous inoculation with the challenge strain of fowl poxvirus.

TABLE 22

RELATION BETWEEN THE TITER OF CHALLENGE STRAIN VIRUS IN SECONDARY LESIONS ON THE COMBS AND THE CLINICAL FEATURES OF THE DISEASE IN CHICKENS INFECTED INTRAVENOUSLY WITH 0.5 ml OF VIRUS

Days After Infection	Titer of Virus PFU/gm	Lesions on Comb	Clinical Features Lesions Elsewhere
1	N	No lesions	No lesions
2	N	"	"
3	6×10^3	"	"
4	3×10^4	"	"
5	2×10^5	Small lesions	Lesions on wattles
6	4×10^7	Papules	"
7	2×10^8	Vesicles	Papules on wattles
8	8×10^7	"	Vesicles on wattles
9	3×10^7	"	"
10	8×10^7	Confluent lesions	On eyes, chest and feet
11	5×10^7	"	"
12	3×10^6	"	"
13 Dead	8×10^6	"	Desiccation of all lesions
14	5×10^6	Desiccation of all lesions	"
15 Dead	3×10^6	Crust formation on lesions	Healing of lesions
16	5×10^6	"	"
17	7×10^6	"	"
18 Dead	3×10^5	Nodules	All lesions healed
19	4×10^6	"	"
20	2×10^7	"	"
21	7×10^5	"	"
23	5×10^4	"	"
35	N	Crusts dropped off	"
60	N	New epithelium formed	"

TABLE 23

RELATION BETWEEN THE TITER OF THE VACCINE STRAIN VIRUS IN THE SECONDARY LESIONS ON THE COMBS AND THE CLINICAL FEATURES OF THE DISEASE IN CHICKENS INFECTED WITH 0.5 ml OF VIRUS

Days After Infection	Titer of Virus PFU/gm	Clinical Features	
		Lesions on Comb	Lesions Elsewhere
1	N	No lesions	No lesions
2	5×10^1	"	"
3	2×10^2	"	"
4	9×10^4	"	Pimples on wattle
5	2×10^5	Small lesions	"
6	2×10^6	Papules	Papules
7	3×10^7	"	"
8	7×10^6	Vesicles	Vesicles
9	6×10^5	"	Lesions on eyelids
10	3×10^6	Confluent lesions	"
11	5×10^6	"	Desiccation of all lesions
12	7×10^5	Desiccation of lesions	"
13 dead	3×10^4	"	Healing of all lesions
14	6×10^5	"	"
15 dead	3×10^5	Crust formation on lesions	"
16	8×10^4	"	"
17	4×10^3	"	"
18	7×10^3	"	"
21	N	Crust dropped off	"
23	N	New epithelium	"
25	N	"	"
35	N	"	"

vaccine strain virus 5×10^1 FPU/gm were detected on the 2nd day. Challenge strain virus was first detected on the 3rd day with a titer of 3×10^3 PFU/gm. Virus was detected in combs 3 days before the visible pox lesions developed on the areas of comb examined for virus content. Virus titers increased logarithmically in the next three days, reaching the maximum titer on the 7th day, when the lesions were fully developed. The titer then remained at maximum level until the 12th day, after which it decreased steadily until after 35 days with challenge strain and 21 days with vaccine strain no virus was detected in the combs of birds. Vaccine strain virus was not detected after the 21st day post-infection which was when the crust dropped off the lesions. The longer persistence of challenge virus correlated with the longer persistence of lesions, but on the 35th day challenge virus was not detected on the combs of chickens which had developed a new epithelium after the crust dropped off the lesions. The growth curves of challenge and vaccine strain viruses in secondary lesions are presented graphically in Figures 10 and 11. Smooth curves have been drawn through the plotted points with some degree of freedom, for the data were derived from different chickens although they were about the same age and were inoculated with the same amount of virus.

5. Multiplication of virus in internal organs and secondary viremia after intravenous inoculation: At intervals of one day, chickens infected intravenously were killed and blood, spleen, liver, kidney, lung and

Figure 10. Growth curve of challenge strain virus in the secondary lesions on the combs of chickens infected intravenously with 0.5 ml of virus (3×10^8 PFU)

Figure 11. Growth curve of vaccine strain virus in the secondary lesions on the comb of chickens inoculated intravenously with 0.5 ml of virus (2×10^7 PFU)

brain samples were tested for virus content to determine the site of virus multiplication and to detect secondary viremia during the development of fowlpox. Tables 12, 13, 17 and 18 show when challenge and vaccine strains of virus were collected in various organs and in the circulation. Both challenge strain virus and vaccine strain virus was detected in lung, liver, kidney and spleen of intravenously infected chickens. However, the titers of virus in internal organs and in the circulation was always much lower than in the comb lesions. Virus was not detected in brain.

The livers and spleens of intravenously infected chickens appeared to be the main organs where virus multiplication occurred. On the first day after inoculation, 6×10^1 PFU/gm of challenge strain virus was detected in the liver. The titer of challenge strain virus in the liver was not constant probably due to individual variation in the reaction of the chickens to the virus. No challenge strain virus was detected in the sample collected on the 5th day, but on the following 3 days the titer was 6×10^2 , 2×10^2 and 2×10^2 PFU/gm respectively. Vaccine strain virus was detected first on the 3rd day in the liver. The highest titer was 8×10^1 PFU/gm when virus was first detected on the 3rd day. Although virus occurred on the 9th day, no virus was detected in the 6th and 8th day samples. Total amount of virus detected in whole liver (Tables 5 and 6) was the highest

TABLE 12

PRESENCE OF VIRUS IN BLOOD AND VARIOUS ORGAN
SAMPLES OF BIRDS INTRAVENOUSLY INFECTED WITH
THE CHALLENGE STRAIN OF FOWLPOX VIRUS

Days After Infec.	Blood				Spleen	Kidney	Liver	Lung	Brain
	Comb	Pla.	RBC	Buffy Coat					
1	N	N	N	N	N	N	P	P	N
2	N	N	N	N	N	P	P	P	N
3	P	N	N	P	P	P	P	P	N
4	P	N	N	P	P	P	P	P	N
5	P	N	N	P	N	P	N	P	N
6	P	N	N	P	P	P	P	P	N
7	P	N	N	P	N	P	P	P	N
8	P	N	N	N	N	P	P	P	N
9	P	N	N	N	N	P	N	P	N
10	P	N	N	N	N	N	N	P	N
11	P	N	N	N	N	N	N	P	N
12	P	N	N	N	N	N	N	N	N
13	P	NT	NT	NT	N	N	N	N	N
14	P	N	N	N	N	N	N	N	N
15	P	NT	NT	NT	N	N	N	N	N
16	P	N	N	N	N	N	N	N	N
17	P	N	N	N	N	N	N	N	N
18	P	NT	NT	NT	N	N	N	N	N
19	P	N	N	N	N	N	N	N	N
20	P	N	N	N	N	N	N	N	N
21	P	N	N	N	N	N	N	N	N
23	P	N	N	N	N	N	N	N	N
35	N	N	N	N	N	N	N	N	N
60	N	N	N	N	N	N	N	N	N

P = Positive for Virus
N = Negative for Virus
NT = Not Tested

TABLE 13
 PRESENCE OF VIRUS IN BLOOD AND VARIOUS ORGAN
 SAMPLES OF BIRDS INFECTED INTRAVENOUSLY WITH
 THE VACCINE STRAIN OF FOWLPOX VIRUS

Days After Infec.	Comb	Blood			Buffy Coat	Spleen	Kidney	Liver	Lung	Brain
		Pla.	RBC							
1	N	N	N	N	N	N	N	N	N	N
2	P	N	N	N	N	N	N	N	N	N
3	P	P	P	P	P	P	P	P	P	N
4	P	N	N	P	P	P	P	P	P	N
5	P	N	N	P	P	P	P	P	P	N
6	P	N	N	P	N	N	N	N	P	N
7	P	N	N	P	P	P	P	P	P	N
8	P	N	N	N	P	P	P	N	N	N
9	P	N	N	P	P	N	P	P	P	P
10	P	N	N	P	N	N	N	N	N	N
11	P	N	N	N	N	N	N	N	N	N
12	P	N	N	N	N	N	N	N	N	N
13	P	NT	NT	NT	N	N	N	N	N	N
14	P	N	N	N	N	N	N	N	N	N
15	P	NT	NT	NT	N	N	N	N	N	N
16	P	N	N	N	N	N	N	N	N	N
17	P	N	N	N	N	N	N	N	N	N
19	P	N	N	N	N	N	N	N	N	N
21	N	N	N	N	N	N	N	N	N	N
23	N	N	N	N	N	N	N	N	N	N

P = Positive for Virus
 N = Negative for Virus
 NT = Not Tested

TABLE 17
SUMMARY OF DATA ON VIRUS DETECTION IN BLOOD AND VARIOUS ORGAN
SAMPLES OF BIRDS INFECTED INTRAVENOUSLY WITH
0.5 ml OF CHALLENGE STRAIN VIRUS (3×10 PFU)

Days After Injection	Blood			Titer of Virus					
	Pla.	RBC	Buffy Coat	Spleen	Kidney	Liver	Lung	Brain	Comb
1	N	N	N	N	N	6×10^1	5×10^2	N	N
2	N	N	N	N	2×10^2	2×10^2	8×10^2	N	N
3	N	N	5×10^1	7×10^2	3×10^2	8×10^1	5×10^2	N	6×10^3
4	N	N	8×10^2	3×10^3	6×10^2	6×10^1	6×10^2	N	3×10^4
5	N	N	5×10^2	N	3×10^2	N	2×10^2	N	2×10^8
6	N	N	1×10^2	2×10^2	6×10^1	6×10^2	8×10^3	N	4×10^7
7	N	N	2×10^2	N	8×10^1	2×10^2	8×10^2	N	2×10^5
8	N	N	N	N	6×10^1	2×10^2	2×10^2	N	8×10^7
9	N	N	N	N	5×10^1	N	8×10^2	N	3×10^7
10	N	N	N	N	N	N	5×10^2	N	8×10^7
11	N	N	N	N	N	N	2×10^2	N	5×10^7
12	N	N	N	N	N	N	N	N	3×10^6
13 dead	NT	NT	NT	N	N	N	N	N	8×10^6
14	N	N	N	N	N	N	N	N	5×10^6
15 dead	NT	NT	NT	N	N	N	N	N	3×10^6
16 dead	N	N	N	N	N	N	N	N	5×10^6
17	N	N	N	N	N	N	N	N	7×10^6
18 dead	NT	NT	NT	N	N	N	N	N	3×10^5
19	N	N	N	N	N	N	N	N	4×10^6
20	N	N	N	N	N	N	N	N	2×10^7
21	N	N	N	N	N	N	N	N	7×10^5
23	N	N	N	N	N	N	N	N	5×10^4
35	N	N	N	N	N	N	N	N	N
60	N	N	N	N	N	N	N	N	N

TABLE 18
SUMMARY OF DATA ON VIRUS DETECTION IN BLOOD AND VARIOUS ORGAN
SAMPLES OF BIRDS INFECTED INTRAVENOUSLY WITH
0.5 ml OF VACCINE STRAIN VIRUS (2×10 PFU)

Days After Injection	Blood			Titer of Virus				
	Pla.	RBC	Buffy Coat	Spleen	Kidney	Liver	Lung	Comb
1	N	N	N	N	N	N	N	N
2	N	N	N	N	N	N	N	5×10^1
3	N	8×10^2	2×10^3	2×10^3	3×10^2	8×10^1	6×10^1	2×10^2
4	N	N	7×10^2	2×10^2	2×10^2	2×10^1	6×10^2	9×10^4
5	N	N	2×10^2	5×10^1	5×10^1	4×10^1	8×10^2	2×10^5
6	N	N	6×10^2	N	N	N	7×10^2	2×10^6
7	N	N	3×10^2	6×10^2	6×10^1	7×10^1	7×10^2	3×10^7
8	N	N	N	5×10^1	4×10^1	N	N	7×10^6
9	N	N	4×10^1	5×10^2	N	3×10^1	8×10^2	6×10^5
10	N	N	2×10^2	N	N	N	N	3×10^6
11	N	N	N	N	N	N	N	5×10^6
12	N	N	N	N	N	N	N	7×10^5
13 dead	NT	NT	NT	N	N	N	N	3×10^4
14	N	N	N	N	N	N	N	6×10^5
15 dead	NT	NT	NT	N	N	N	N	3×10^5
16	N	N	N	N	N	N	N	8×10^4
17	N	N	N	N	N	N	N	4×10^3
19	N	N	N	N	N	N	N	7×10^3
21	N	N	N	N	N	N	N	N
23	N	N	N	N	N	N	N	N

TABLE 5

WEIGHTS (IN GRAMS) OF INTERNAL ORGANS OF BIRDS INFECTED
INTRAVENOUSLY WITH THE CHALLENGE
STRAIN OF FOWLPOX VIRUS

Age of Birds	Days After Infection	Spleen	Kidney	Liver	Lung	Brain
25 days	1	0.4	2.6	6.8	1.8	-
26	2	0.8	3.2	12.4	2.8	1.3
27	3	0.9	3.6	9.6	2.2	1.6
28	4	1.3	3.9	13.4	2.3	1.7
29	5	1.0	3.8	12.0	3.2	1.7
30	6	2.0	3.5	12.0	2.6	2.0
31	7	1.1	3.8	14.0	2.4	2.0
32	8	0.7	4.6	10.5	2.8	2.0
33	9	0.7	4.2	11.6	2.4	2.0
34	10	1.0	4.3	14.6	3.0	2.0
35	11	0.9	4.5	13.4	2.8	2.1
41	12	1.1	3.4	12.6	3.4	1.3
42*	13	0.5	4.2	14.3	3.8	2.0
43	14	1.2	5.1	13.5	3.8	1.9
44*	15	0.7	4.4	14.5	4.2	2.1
45	16	1.2	4.8	16.0	3.8	2.0
46	17	1.2	4.9	15.4	4.0	2.1
47*	18	0.3	5.2	18.0	4.2	2.0
48	19	0.9	4.5	16.6	4.9	2.3
49	20	1.1	5.2	19.5	5.3	2.0
50	21	1.7	5.9	17.6	4.6	1.9
52	23	1.6	7.0	21.0	4.8	2.3
64	35	NW	NW	NW	NW	NW
79	60	NW	NW	NW	NW	NW

* Dead

TABLE 6
 WEIGHTS (IN GRAMS) OF INTERNAL ORGANS OF BIRDS INFECTED
 INTRAVENOUSLY WITH THE VACCINE
 STRAIN OF FOWLPOX VIRUS

Age of Birds	Days After Infection	Spleen	Kidney	Liver	Lung	Brain
30 days	1	0.8	3.2	13.6	3.6	1.4
31	2	1.0	2.8	12.4	3.2	1.5
32	3	0.9	3.7	14.2	3.7	1.85
26	4	0.7	2.8	10.4	2.6	1.7
27	5	0.85	3.2	12.4	2.8	1.8
28	6	0.7	3.0	13.0	2.8	1.8
29	7	0.9	2.6	13.6	3.6	1.7
30	8	0.8	3.0	12.4	3.2	1.9
31	9	0.7	3.2	13.0	2.8	1.8
32	10	0.9	3.6	12.8	3.2	2.0
33	11	1.0	4.2	14.3	4.0	2.0
34	12	0.7	2.6	12.6	3.4	2.0
42*	13	1.6	4.0	15.0	4.3	2.0
43	14	1.6	4.2	17.8	3.5	1.9
44*	15	2.0	4.6	19.3	3.8	2.1
45	16	1.3	4.6	16.6	3.4	2.0
46	17	1.2	4.8	22.3	4.5	2.0
48	19	1.3	4.2	24.8	5.2	2.1
50	21	1.5	4.6	24.5	4.8	2.0
52	23	1.4	5.8	18.6	4.2	2.2

* Dead

comparing the concentration of virus in whole spleen, kidneys and lung.

There was a difference between challenge strain virus and vaccine strain virus levels in spleens. Challenge strain virus was detected on the 3rd day (Table 11). The titer was 7×10^2 PFU/gm and increased on the next day to 3×10^3 PFU/gm. Challenge strain virus was not detected on the 5th day, but titer was again 2×10^2 PFU/gm on the 6th day when the virus was last detected in the spleens. On the 3rd day the titer of vaccine strain virus in the spleen was 2×10^3 PFU/gm and in the next 2 days the titer decreased (Table 18). It was not detected in the 6th day spleen sample. In samples collected on the following 3 days the titers of virus were 6×10^2 PFU/gm, 5×10^1 PFU/gm and 5×10^2 PFU/gm respectively.

Challenge strain virus persisted in the lungs until the 11th day after inoculation (Table 17). The titer increased to the 6th day, reaching the maximum titer of 8×10^3 PFU/gm, and remained high on 7th and 8th days. The titer of challenge strain virus was 2×10^2 PFU/gm when the virus was last detected in lungs on the 11th day post-infection. Vaccine strain virus was not present in samples collected one and two days post-inoculation, but reappeared in the lungs at the low titer of 6×10^1 PFU/gm on the 3rd day. The titer reached 6×10^2 PFU/gm on the 4th day, and remained almost at this titer until it was last detected in lungs on 9th day post-infection (Table 18).

Challenge strain virus was first detected in the kidneys on the 1st day. The highest titer of virus was 6×10^2 PFU/gm on the 4th day and the titer fell steadily until it was not detected on 9th day post-inoculation (Table 17). Vaccine strain virus levels were similar to challenge strain virus levels in the kidneys (Table 18).

During the viremic stage which lasted from the 3rd day through the 7th day the challenge strain virus was detected only in the buffy coat fractions of the blood. The highest titer of virus was 8×10^2 PFU/gm on the 4th day post-inoculation (Table 17). Vaccine strain virus was detected in the circulation from 3rd day through 9th day. On the 3rd day, when vaccine strain virus reappeared in circulation, 8×10^2 PFU/ml were detected in the RBC fraction and 2×10^3 PFU/ml were found in buffy coat fraction of blood. From the 4th day onward, the vaccine strain virus was present in small quantities and was associated with the buffy coat fraction only (Table 18). These results are graphically shown in Figures 12 and 13.

6. Macroscopic examination of internal organs of intravenously inoculated chickens: No macroscopic change was evident in the internal organs except for the spleen at autopsy. Kidneys, livers and lungs appeared to be normal during the development of fowlpox in intravenously infected

Figure 12. Concentration of challenge strain virus in the comb and in the blood, spleen, kidneys and lungs of chickens inoculated intravenously with 0.5 ml virus

Figure 13. Concentration of vaccine strain virus in the comb and in the blood, spleen, kidneys, liver and lungs of chickens inoculated intravenously with 0.5 ml virus

chickens. Spleens were slightly enlarged and congested between the 6th day and 12th day (Table 5). At the peak of the severe disease in some chickens the spleens were obviously small and dark brown in color. The spleens from the recovered chickens appeared normal.

C. Experiment III. Intratracheal inoculations (IT)

Of the 30 chickens infected intratracheally with 0.05 ml of challenge strain virus, none developed lesions on the combs and wattles or on any other parts of the body. Two birds were killed daily for samples until the 10th day post-inoculation. Fractions of blood and samples from each chicken were prepared. Virus was completely absent from the blood, indicating that there was no viremia in the chickens infected intratracheally. Spleen, kidney and liver were found to be free of virus in all samples tested (Tables 14 and 19). The only evidence of multiplication of virus was in the lung samples. Virus was not detected in the lungs until the 3rd day post-inoculation. The amount of virus detected in the lungs from the 3rd day through the 7th day was small (Figure 14).

In general, the condition of chickens inoculated intratracheally remained normal. Thirty-five days after the original intratracheal injection of virus, two of the chickens were challenged intravenously with 0.5 ml

Figure 14. Growth curve of challenge strain virus in the lungs of chickens infected intratracheally with 0.05 ml of virus

TABLE 14

PRESENCE OF VIRUS IN BLOOD AND VARIOUS ORGAN
SAMPLES OF BIRDS INFECTED INTRATRACHEALLY WITH
THE CHALLENGE STRAIN OF FOWLPOX VIRUS

Days After Injection	Blood					Spleen	Kidney	Liver	Lung
	Comb.	Pla.	RBC	Buffy	Coat				
0*	N	N	N	N		N	N	N	N
1	N	N	N	N		N	N	N	N
2	N	N	N	N		N	N	N	N
3	N	N	N	N		N	N	N	P
4	N	N	N	N		N	N	N	P
5	N	N	N	N		N	N	N	P
6	N	N	N	N		N	N	N	P
7	N	N	N	N		N	N	N	P
8	N	N	N	N		N	N	N	N
9	N	N	N	N		N	N	N	N
10	N	N	N	N		N	N	N	N
11	N	N	N	N		N	N	N	N
12	N	N	N	N		N	N	N	N
13	N	N	N	N		N	N	N	N
14	N	N	N	N		N	N	N	N
15	N	N	N	N		N	N	N	N
16	N	N	N	N		N	N	N	N

P = Positive for Virus

N = Negative for Virus

* = Killed 3-4 minutes after injection

TABLE 19
TITERS OF VIRUS IN LUNGS OF CHICKENS INFECTED
INTRATRACHEALLY WITH 0.05 ml OF
CHALLENGE STRAIN VIRUS (3×10 PFU)

Birds Examined On Day After Infection	No.	Titer of Virus*	Clinical Feature
0	1	N	No lesion
1	2	N	"
2	2	N	"
3	2	3×10^2	"
4	2	4×10^2	"
5	2	3.5×10^2	"
6	2	3×10^1	"
7	2	4×10^1	"
8	2	N	"
9	2	N	"
10	1	N	"
11	1	N	"
12	1	N	"
13	1	N	"
14	1	N	"
15	1	N	"
16	1	N	"

of challenge strain virus and another 2 chickens were challenged intradermally with large doses of challenge strain virus on extensively scarified combs. None of these chickens developed any sign of disease. The results of the test for immunity revealed that the intratracheally-introduced virus produced a good immunity without causing development of any lesions.

V. DISCUSSION AND CONCLUSIONS

The understanding of the pathogenesis of fowlpox, as of all infectious diseases, consists of a knowledge of the routes of invasion, spread of the virus in the body and the ultimate fate of the virus as the infected animal recovers and becomes immune, or as it is overwhelmed by the invasion.

Fenner (1948) has proposed a general hypothesis to explain the sequence of events in pox diseases. In experimental studies of ectromelia in mice, Fenner found that mice infected with small doses of ectromelia virus developed a local lesion at the site of inoculation with rapid involvement of the regional lymph nodes. The virus multiplied at the site of entry and in the regional lymph nodes and then entered into the circulation resulting in spread of virus to the reticulo-endothelial system in general and to the liver and spleen in particular. Multiplication of the virus in these organs led to overflow of virus into the circulation which caused further generalization with involvement of the skin. A similar pathway of infection was found in myxomatosis in rabbits (Fenner and Woodroffe, 1953). In both ectromelia and myxomatosis, infection

takes place through the skin and there is a definite primary lesion followed by a detectable viremia. This sequence of events is not readily observed in experimental fowl pox infection. The following discussion will be restricted to the routes of infection we used in our experiments and the sequence of events detected in experimentally produced fowl pox infections.

The skin is the site of the primary lesions in many pox virus infections. For instance, breaks in the skin of mice may be infected by ectromelia (Fenner, 1948) and the skin of rabbits is the primary site of infection in myxomatosis (Fenner and Woodroffe, 1953) and in rabbit pox (Bedson and Duckworth, 1963). When variolation was practiced before the vaccinia virus came into general use for immunization for smallpox, the inoculation of smallpox matter by incision or puncture of the skin produced a local lesion (Downie, 1965; Downie and Dumbell, 1956). Vaccinia virus is today introduced into the skin of man in vaccination against smallpox.

It is generally believed that fowl poxvirus causes infection through contact of virus with scratches or wounds on the comb and wattles or on other unfeathered parts of the body. The results obtained in this study in chickens infected intradermally with challenge, outbreak and vaccine strains of fowl poxvirus supported the hypothesis that at least the

invasion of cells of the skin is initiated when virus is deposited on the scarified comb of chickens. Although multiplication of virus at the site of inoculation resulted in local lesions, viremia and signs of virus in the internal organs were not detected in 77 of the 80 chickens infected intradermally. No secondary lesions were seen in any of the birds observed during the experiment. The results of these experiments show that in only a few of the chickens infected intradermally did virus reach the internal organs in detectable amounts. Development of secondary lesions to a limited extent following the scarification of fully developed lesions has been reported (Goodpasture, 1928) and some workers have observed slight secondary lesions on the head in less than 5 percent of chickens vaccinated with virulent fowl poxvirus (Beaudette, 1941). The results of this experiment suggest that in the development of a typical fowl pox outbreak in nature, some other factors, such as management or the presence of internal parasites, may play a more important role than just the contamination of small injuries with the limited number of fowl poxvirus particles available in the environment.

The sequence of events described following intradermal ectromelia infection (Fenner, 1948) was not observed in the chickens we infected intradermally with fowl poxvirus. Isolation of virus particles, if any, in the blood stream following intradermal infection is usually impossible.

for the few particles which enter the circulation are probably taken up rapidly by the macrophages of the liver and spleen. Virus may be isolated from the internal organs of some birds as a result of multiplication of virus which passes the macrophage barrier and initiates the infection in the cells of the organs (Mims, 1959, 1964). In chickens infected intradermally it is more likely that even if the virus reaches the internal organs and initiates infection and multiplies there the damage caused by virus multiplication in these organs rarely causes any fatal effect. Virus finds its way to the skin to develop secondary lesions in only very few chickens.

An interesting finding in this study was the growth curve of the fowl poxvirus in the primary lesions which developed following the intradermal inoculation. Of the approximately 3×10^7 PFU of challenge virus deposited on the scarified comb only 2×10^2 PFU were detected in the inoculated area of the combs of chickens killed 3 to 4 minutes after inoculation. Titration for virus was made on the same day which the sample was collected. Virus was not detected on the comb in amounts comparable to the amount inoculated until the 2nd day post-inoculation. This indicated that most of the virus deposited on the comb disappeared. Thus, most of the virus detected in the comb represented the new virus particles,

the titer of which increased logarithmically between the 2nd and the 8th day. The growth curve of fowl pox in the comb resembled the growth curve of poxviruses reported to occur in tissue culture (Joklik and Rodrick, 1959; Furness and Younger, 1959)(Figures 2 and 3).

Other than that, there is development of inclusion bodies very little is known about the growth of fowl poxvirus in skin cells. The extensive literature concerning the nature of the cell types involved in the primary lesions of vaccinia and variola has been reviewed by Van Rooyen and Rhodes (1948). The lesions have been considered to be essentially epidermal, dermal and vascular. Although epidermal cells are among those primarily infected, dermal cells are more important for the initiation of infection by ectromelia. Roberts (1962) studied the sequence of events in primary lesions using the direct fluorescent antibody technique and titration method in serial sections of mouse skin infected with ectromelia and found that both epidermal and dermal cells were involved in the earliest stages. No attempt was made, in this experiment, to determine in which part of the comb virus multiplication took place. Only in one comb collected on the 9th day post-inoculation the virus content of dermis and epidermis containing the lesions was titrated in separately prepared parts of the comb. Four $\times 10^2$ PFU were detected in the dermal part and 3×10^5 PFU were found in the epidermal part of the comb. This indicated that in fowl pox, as

in ectromelia, both the dermal cells and epidermal cells are involved in multiplication of virus in the comb. In order to follow the development of primary lesions and discuss the role of intradermal infection in the spread of virus in the body, close study of primary lesions by fluorescent antibody technique would provide useful information.

Reticuloendothelial macrophages, particularly those in the liver and spleen, are capable of rapidly clearing the blood stream of intravenously injected particles (Biozzi et al., 1953; Erickson et al., 1957; Brunner, 1960). Clearance of poxviruses from the blood has also been investigated and vaccinia virus was reported to be cleared much more rapidly than are smaller viruses (Mims, 1959, 1964).

The significant feature of fowlpox virus clearance was that the virus disappeared from the circulation very rapidly. The titer of virus in the blood fell rapidly within the first 4-5 minutes during which time about half of the injected virus was cleared. In the next 3-4 hours, most of the remaining virus was cleared from the circulation. Thereafter, there was no further decrease in the amount of virus in the blood within the next 4-5 hours. During this latter period probably some of the virus cleared from the circulation by the liver and spleen was again released and entered into the circulation. Virus was associated with cellular components of the blood. Although the titer of virus detected in the red cell fraction was almost the same as in the buffy coat fraction during

the first 3 hours of clearance, the shift of virus from the red cell fraction to the buffy coat fraction was significant after the first 3 hours. Virus was very largely absent from the plasma. The results suggest that virus was associated with cells of the white cell series, but no attempt was made in this experiment to determine which of the various cell types was involved. According to Fenner and Woodroffe (1963) the virus detected during the viremia stage in myxomatosis in rabbits is associated with lymphocytes rather than granulocytes.

The virus which disappeared from the circulation was detected in the liver, spleen, kidney and lung of the chickens which were killed up to 15 hours post-inoculation. Almost 15 times more virus was detected in the whole liver than was detected in the whole spleen. This result was expected because, in birds, the mass of the reticulo-endothelial system is concentrated in the liver. Virus disappeared from most internal organs examined within 13 hours. The only exception was that in the lungs of chickens infected with challenge strain virus, virus was detected in samples collected 23 hours after inoculation. The disappearance of virus from internal organs, especially from the liver and spleen has been interpreted as a result of intracellular digestion and destruction of fowl pox virus in these organs (Mims, 1959).

The amount of virus which enters into the blood stream during natural infection must be small in comparison to the large amount of virus inoculated intravenously. The rapid clearance of intravenously injected large amounts of fowl pox virus from the circulation and its destruction in the liver and spleen in a short time explains why following intradermal infection, the detection of virus in the circulation and internal organs is rare.

The most interesting result of the intravenous inoculations was that a disease similar to natural infection developed in all chickens following intravenous inoculation of fowl poxvirus. The sequence of events described in ectromelia following intradermal infection (Fenner, 1948) was observed in chickens following the intravenous inoculation of virus except that the stage of invasion from the primary site of infection on the skin and the primary viremia were eliminated.

The secondary lesions which developed on the comb, wattles and other less feathered parts of the body were similar to the lesions described in natural outbreaks (Hutyra, et al., 1945). The growth of fowl pox in the secondary lesions showed that the virus detected in combs was distributed to these regions via the blood when the virus was injected intravenously. Virus localized in the cells

of the comb was not detected until the 2nd day post-inoculation (Figures 10 and 11). This showed that the lag phase followed by a logarithmic increase is the same in combs whether the virus is deposited on the injured comb or carried there by the circulation.

Another interesting finding was that when vesicles formed in comb lesions, the amount of virus detected in the combs was at its maximum level of about 10^8 PFU/gm (Tables 22 and 23). In ectromelia, this stage of disease is characterized by the liberation of large amounts of virus into the environment and Fenner (1948) considered that this period is the most infectious. In fowl pox, because of the impermeable nature of the upper layer of skin, virus is not shed to the surface and lesions are not infectious under normal conditions at this stage. But, it is obvious that if these lesions are broken, the amount of virus which would be shed into the environment would be much greater than would be shed in scabs in which the titer of virus is much lower.

A viremic stage was demonstrated in all intravenously infected chickens. Virus was detected first 2 days after the clearance of virus from the circulation and from the internal organs. Virus was present in the buffy coat fraction of the blood between the 3rd and 8th day post-inoculation, but the titer never reached more than 3×10^3 PFU/ml

at maximum level. There was a relation between viremia and multiplication of virus in internal organs. Because the growth curves of virus in combs shows that the virus is abundant in comb lesions at the time viremia is detected, some writers think (Dhillon, 1966) that virus is probably shed from the skin into the blood and persists there in low quantities until the virus is eliminated from the skin. The author believes that virus detected in the blood was shed from the internal organs where the virus multiplication took place. The growth curve of primary and secondary lesions indicates that almost the same amount of virus is present in combs whether the combs are infected intradermally or through the blood stream. If the virus in the circulation originated from the comb lesions, the same amount of virus should be detected in blood of intradermally infected chickens as in intravenously infected ones.

The part played by the internal organs, i. e. , spleen, liver, kidney and lung is of interest. In the study of the development of the disease, first the accumulation in, then the disappearance of virus from these organs within 13 hours was demonstrated. The reappearance of virus indicates that not all of the virus taken up by the cells of these organs was destroyed and that some of the virus particles invading cells of the organs initiated multiplication. During the period of multiplication of

virus, the amount of virus in the internal organs per gram was always lower than was found in combs. No macroscopic change was observed at autopsy in kidney, lung and liver but the spleens of chickens overwhelmed by infection were smaller than normal (Tables 1-7). According to Fenner (1948) macroscopic signs of infection appear on the skin and in the internal organs when the titer reaches a level of 10^7 PFU/gm. Although virus was detected in the internal organs of chickens in this experiment, none of the organs at any time had a titer close to 10^7 PFU/gm.

Another interesting finding was that there was no virus in the internal organs examined after 13 days post-inoculation whether the chickens were overwhelmed by the infection or recovered. The virus always disappeared from the internal organs long before the virus was eliminated from the combs by the healing process.

Although macroscopic change was seen in spleens of some chickens overwhelmed by infection, no histological examination was made of these organs. The highest total amount of virus was detected in the liver and in spite of there being no macroscopic changes, damage due by virus multiplication in liver cells was probably the most important factor causing death. Although the spleen is not a vital organ, as is the liver,

virus particles invading the spleen cells may grow and produce tissue damage which may interfere with antibody production.

There is no satisfactory information about the accumulation of virus in lungs and kidneys except that there seems to be a specific affinity of fowl poxvirus for the cells of these organs. The reappearance of virus in the kidney and lung may be the result of multiplication of virus localized in cells or accumulation of new virus particles during viremia. It is interesting to note that kidney cells are of main interest to tissue culture workers. Bethula and Matthews (1962) attempted to grow fowl poxvirus in the cells of chicken embryo kidney and found that fowl poxvirus grew in kidney cells, but failed to show any cytopathic effect.

Infection by inhalation has long been suspected as an important means of transmission of a number of poxviruses (Roberts, 1962). Hahon and Wilson (1960) produced typical disease in monkeys with airborne variola virus and though there is no direct evidence, in smallpox it is considered that virus normally enters by the respiratory tract.

The results of this experiment showed that the multiplication of fowl pox in lungs was not followed by a detectable viremia. Virus neither was detected in other internal organs, nor were secondary lesions observed in

chickens infected intratracheally. Francis (1956) reported a similar observation in chickens exposed to airborne fowl poxvirus. Viremia and secondary lesions were not seen in any of the chickens although about 10^3 PFU of virus per gram was detected in the lungs. It is more likely that viremia and detection of virus in other parts of the body depend on the degree of infection. Hahon and Wilson (1960) detected up to 10^7 PFU of virus per gram in lungs of monkeys. The infection of the lung was followed by a detectable viremia and skin eruptions. Francis (1956) also reported the death of some chickens and the development of slight secondary lesions in a group of chickens after heavy doses of airborne fowl pox but in this experiment the virus content of lungs was not determined. Results of intratracheal injection by Francis, suggest that infection may spread by the respiratory route in certain conditions. However, in nature many chickens may become immune following respiratory infection without developing a serious disease or visible lesions.

There is no satisfactory information about how fowl pox virus is distributed in the body in natural infections. Some workers believe that fowl poxvirus multiplies first in the epidermal cells at the site of entry and from these local lesions enters the blood stream and is carried to further parts of the skin and internal organs (Sanfelice, 1914; Doyle and

Minett, 1927; Goodpasture, 1928; Francis, 1950; Dhillon, 1966), and one group has reported that the strain of fowl poxvirus they studied forms only localized lesions at the site of entry without generalization (McGauphey and Burnett, 1945). The presence of virus in the circulation following intradermal inoculation has been reported (Sanfelice, 1914; Doyle and Minett, 1927), but no details were given about the stages of the disease when virus was detected in the circulation. Detection of virus in the blood of 2 birds (Dhillon, 1966) or development of slight secondary lesions in a few chickens (Francis, 1956) does not provide enough data to draw general conclusions about the role of the blood in dissemination of fowl poxvirus. Although disease similar to naturally occurring fowl pox has been produced by intravenous inoculation (Burnet, 1906; Dhillon, 1966) and virus has been demonstrated in the blood after intradermal infection during the development of the local lesions (Doyle and Minett, 1927; Dhillon, 1966) the pathways of spread of fowl poxvirus in natural infections are still unclear.

In this study the clinical features of the disease, the fate of virus after inoculation and pathways of infection were studied in about 250 chickens infected with one or the other of three strains of fowl poxvirus differing in their virulence. Intradermal, intravenous and intratracheal

routes of infection were studied comparatively. Samples taken at intervals after infection were examined for the presence of virus, and, if there was any, the amount of virus in the sample was determined by the pock counting method so that growth curve of virus in various organs could be plotted. It is impossible to be certain how far the findings in these experiments are applicable to naturally occurring fowl pox infection, but information obtained in each experiment does give a general idea about pathogenesis of fowl pox.

The results of our studies of these various routes of infection indicate that fowlpox may enter the body by a wide variety of routes. Moreover, in the artificial conditions of laboratory infection typical local lesions were induced by depositing the fowlpox virus on an injured part of the comb; typical fowl pox with development of generalized lesions was only produced by the intravenous route and at least immunity was demonstrated in chickens following instillation of virus into the lungs. In nature, however, it is probable that only some of these routes or only a single route predominate in fowl pox infection. The routes of infection in natural fowl pox disease are determined by circumstances. Insect vectors, airborne virus and contamination of skin may all be important in particular circumstances.

Whatever the routes of infections are, and however viruses differ in virulence, for typical fowl pox to occur in nature, fowl poxvirus must reach the internal organs such as the liver and spleen and invade the cells of these organs. The disease is fatal when the concentration of virus in any organ reaches the level of 10^7 PFU per gram (Fenner, 1948). Macroscopic signs of infection appear in the skin only when the titer reaches this level. However, development of skin lesions is not followed by death of chickens in many cases when the vital organs are not damaged by virus multiplication and this explains how, in nature, chickens develop immunity with or without signs of lesions on the skin. Virus titers of more than 7×10^3 PFU per gram were not detected in any of the internal organs of the chickens which recovered and became immune after the disappearance of virus from skin. In the case of intratracheal injection, chickens were immune to virus without developing any lesions on the skin. It is noteworthy that development of local lesions or secondary lesions on the skin with great virus concentrations has no effect on the general condition of chickens. Severe disease develops following damage to internal organs, especially liver and spleen, which interferes with the vital function of these organs. Reticuloendothelial cells are important in the initiation and control of virus spread following contact of chickens with virus.

The development of disease conditions similar to those observed in natural fowl pox outbreaks only after the intravenous injection of virus suggests that the critical level of 10^7 PFU per gram in internal organs and skin may be achieved in natural outbreaks when large quantities of virus enter into the circulation. Transfer of virus from vesicles where the virus is found free and abundant, directly into the blood stream of chickens by mosquitoes could create such a condition. The growth curve of virus in primary lesions reveals that following dermal infection the vesicles developed at the sites of infection could serve as a good source of virus for the spread by mosquitoes.

Infection by the respiratory route may be important under certain conditions. When the virus multiplication in the lungs is not controlled by lung macrophages, an overflow of virus into the blood stream may result in infection of other internal organs and skin and death. Probably this route of infection produces undetectable disease in nature and in most cases results in development of immunity.

The growth curve of viruses in primary lesions, in secondary lesions and in internal organs indicates that virus disappears in internal organs first, then in comb lesions, thus there is no carrier state after chickens recover.

The results of these experiments also indicate that the primary viremia is the most important part of infection in development of generalized lesions on skin and infection of internal organs. This study suggests that the pathogenesis of fowl pox needs to be more carefully studied to determine the routes of infection in nature and the sequence of events especially concerning the infection of internal organs during the development of the disease.

These studies also indicate that factors other than exposure of chickens to virus are important in the development of typical fowl pox. Further, study should be directed to the consideration of host resistance factors and to the role of insects in infection.

The immune fluorescent technique has great advantages in studies of virus pathogenesis. The precise detection of virus in the site of initial invasion and determination of the fate of virus in local lesions and internal organs by the fluorescent antibody technique along with clinical and epidemiological observations of natural disease with detection and titration of virus in blood and in internal organs of chickens in various stages of fowl pox during a natural outbreak could provide important information regarding the pathogenesis of fowl pox.

VI. SUMMARY

A comparative study of the pathogenesis of experimental fowl pox in chickens infected with challenge, outbreak or vaccine strains of fowl pox by three routes has been made. Chickens were infected by the intradermal, intravenous or intratracheal routes and the spread of virus within the body was traced by daily titration of samples collected from the chickens. Chorioallantoic membranes of 12 day old developing chick embryos were used by the drop membrane method for the detection of virus in samples.

Chickens were infected intradermally with the challenge strain, outbreak strain or vaccine strain of fowl poxvirus. Each strain of virus following intradermal inoculation produced a similar clinical feature. The lesions developed at the site of inoculation only. The lesions became evident on the scarified areas of the combs on the 4th day. These lesions went through the typical stages of development of fowl pox. Following the papular and vesicular stages, the lesions became desquamated. Healing took place without cicatrization. Except that there was a shorter time for the development of new epithelium

over lesions produced by the vaccine strain than by the challenge and outbreak strain viruses, the strain of virus had little effect on the appearance of primary lesions. The general condition of the birds remained normal. Virus was not detected in the blood or internal organs in 77 of the 80 chickens infected intradermally.

The characteristic clinical feature of the disease in the intravenously infected chickens was the development of secondary lesions. Typical pock lesions developed on the comb, wattles, eyelids and skin of the birds infected with challenge strain or vaccine strain virus. The disease which developed following intravenous inoculation was more severe than that which developed following intradermal or intratracheal inoculation. Five birds of inoculated died 13 to 18 days post-inoculation in two experiments. The sequence of events described in ectromelia was observed in chickens following the intravenous inoculation of virus except that the stage of invasion from the primary site of infection on the skin and the primary viremia were replaced by the inoculation. Virus was detected in liver, spleen, kidney and lungs, but not in brain. The liver and spleen appeared to be the main organs where virus multiplication occurred. During the viremia which lasted from the 3rd through 7th day virus was detected in the buffy coat fraction of the blood only.

In general, the chickens inoculated intratracheally with challenge strain virus remained clinically normal. Virus was absent from the blood, liver, spleen and kidney. Small quantities of virus were detected in the lungs from the 3rd day through 7th day. No secondary lesions were observed.

The results of these experiments indicated that fowl pox virus may enter the body by a wide variety of routes. Intradermal and intratracheal routes of infection result in development of immunity without signs of a serious disease. Typical fowl pox with the development of generalized lesions were produced by the intravenous route only. It is doubtful that massive intravenous infection occurs in nature, thus host resistance or other factors still not understood must be important in natural outbreaks of fowl pox.

Disappearance of virus from the circulation and from the internal organs was studied in the chickens infected intravenously. Virus disappeared from the circulation very rapidly. About half of the virus was cleared from the circulation within the first 4-5 minutes. Virus was associated in the blood, mainly with the buffy coat fraction. Virus was not detected after the 15th hour in the circulation. The virus which disappeared from the blood was detected in the liver, spleen, kidney

and lungs of the chickens. Almost 15 times more virus was detected in the whole liver than was detected in any other organ. Destruction of virus in the cells of liver and spleen was rapid; virus disappeared from most internal organs within 13 hours.

Growth curves were determined for challenge and vaccine strain viruses by daily titration of virus in the comb and in internal organs. The growth curve of virus in the primary and secondary lesions was almost the same. The lag phase and logarithmic phase was demonstrated in comb lesions. The greatest amount of virus was detected in the combs when the vesicles had formed. Titer decreased when the crust formed and virus was not detected in comb after new epithelium developed. Virus content of spleen, liver, kidney and lung was always lower than in comb lesions. Virus disappeared from internal organs before it did from comb lesions.

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