CHAPTER 1
INTRODUCTION

a) General Aspects of the Disease caused by Infection with the Australian Infectious Bronchitis Virus:

Prior to 1962, infectious bronchitis (IB) virus had not been identified in Australia. Certainly the disease syndrome caused by IB virus, as seen in Europe and America (Beister and Schwarte, 1959) had not been described in Australia (Gilchrist, 1962, Hungerford, 1962), although reports by Hart (1946), Newton and Simmons (1963) and Gilchrist (1962), all describe syndromes which had some aspects reminiscent of IB infections. One of the prominent poultry diseases at that time had been characterised by a breakdown in kidney function producing a 'uraemia' or nephritis (Hungerford, 1969).* This syndrome had been seen since 1948 and was thought possibly to be caused by some nutritional factor (Beilharz and McDonald, 1960). In 1962, Cumming isolated a virus from cases of nephritis and suggested the virus to be IB. This was confirmed by Cumming

*N.B. The term uraemia, uricaemia, nephrosis and nephritis have been used interchangeably by many authors working with the Australian IB virus. Only the term 'nephritis' will be used in this thesis.
(1963, 1964), Gilchrist (1963), and Gilchrist and Sinkovic (1964). Supporting serological evidence for the identity of the virus being IB was given by cross neutralisation studies with American IB viruses (Winterfield et al., 1964a), British IB viruses (Berry and Stokes, 1968) and some German IB viruses (von Bulow, 1967). Berry and Stokes (1968) also confirmed the Australian IB virus as a corona-virus by electron microscopy.

The Australian viruses, as well as producing fairly typical IB respiratory symptoms, also produce the nephritis syndrome. However, even as late a 1964, Pryor and Woo described a nephritis of young chicks, seemingly attributable to a nutritional factor. Cumming (1967a) is at pains to point out a number of differences that characterised the disease entities described by both Beilharz and McDonald (1960) and Pryor and Woo (1964) from the nephritis caused by the Australian IB virus. It would seem that there are other causes of nephritis besides IB virus and some caution is needed in diagnosis when nephritis is present (vide Hungerford, 1962, 1969).

The disease syndrome caused by the Australian isolates of IB virus has been adequately reviewed by Cumming (1969a) and Hungerford (1969). As such, the main facts of the disease will be outlined here
without further reference.

The course of the disease in four-week-old birds, when infected by the infra-orbital instillation of infected allantoic fluid, is first seen as a transient gasping and coughing within 24 hours. This is followed, at about six days after infection, by the birds ailing, and dying from a nephritis characterised at post-mortem by swollen, creamy coloured kidneys, the tubules of which are distended with urates.

Morbidity and mortality, due to the nephritis phase, rises to a peak at 10-14 days after infection and then subsides, little sign of distress being seen four weeks after infection. At the same time as the nephritis phase, there is a marked increase in water consumption that may last for five to six weeks and which results in very wet droppings. Food intake during this phase may also be reduced. The recovered birds are resistant to recurrence of the disease syndrome when re-infected by the same virus. If the outbreak occurs when the birds are in lay, a marked drop in egg production is seen, seven to eight days after infection. Egg production may return to normal in three to four weeks, but shell quality remains poor for a longer period of time. There may also be an affect on internal egg quality (Cumming, 1965)
Several factors may affect mortality. There are marked differences in the virulence of virus strains and mortality can vary under set conditions from 1 - 20% or more, although all viruses so far tested have been serologically related. Ambient temperature affects the severity of the disease outbreak markedly, cold (16°C) causing an increase in mortality and warmth the reverse. There are both sex and breed differences in susceptibility, males being twice as susceptible as females and White Leghorns more susceptible than other breeds. Higher mortalities have been recorded in birds on meatmeal based diets compared with those on soybean based diets.

The actual cause of death seems to be the inability of the affected kidney to re-absorb sodium and potassium ions. This can occur as early as 48 hours after infection. This loss of ions can be made up by the use of electrolyte replacers in the drinking water (Heath, 1967, 1968, 1970; Cumming and Heath, 1969).

Treatment of an outbreak is best achieved by an increase in ambient temperature and, if given before the nephritis phase, electrolyte replacers in the drinking water. Preventative treatment, using live virus vaccines, is now the more usual course of action in the field.

Winterfield et al., (1964a), von Bulow (1967)
and Berry and Stokes (1968) all showed that sera formed against Australian viruses will neutralise a number of European and American isolates. American antisera did not neutralise the Australian viruses tested (Winterfield et al., 1964a) although some of the British sera (Berry and Stokes, 1968) were able to neutralise an Australian virus. Despite some variation, it would seem that all the Australian viruses so far tested are closely antigenically related (Cumming 1967b; Stephens and Simmons, 1968; Westbury, 1970)

Serological surveys in New South Wales by Cumming (1967b; 1969b) and by Stephens and Simmons (1968) in Queensland, indicated that the majority of flocks had neutralising antibodies to IB virus. Cumming also produced evidence that Mycoplasma gallisepticum negative flocks were more likely to be free of IB virus antibodies for a longer period of time than M. gallisepticum positive flocks. Once IB antibodies appeared, however, all the flocks on the farm became IB positive quickly, indicating rapid spread of the infection. Flocks on the farms then remained positive for IB antibodies for at least several generations.

Stephens and Simmons (1968), followed a field flock for neutralising antibodies and showed a decline in the number of birds with passive antibody by four weeks of age. Neutralising antibody had re-appeared by ten weeks
of age and was easily detected thereafter to 18 months of age.

Cumming (1967b) showed that one to four day old chicks with maternal antibody were less susceptible to the disease syndrome, although still susceptible to virus infection. He also showed that birds recovered from infection were refractory to infection with the same virus. Cumming (1969a) reported that Hofstad had shown that birds immunised with a Massachussets isolate of IB virus were resistant to infection with the Australian T virus.

A number of overseas isolates of IB virus have been cultivated in tissue culture, the isolate most frequently cultivated being the highly egg adapted Beaudette strain (see Coria, 1969; Cunningham, 1970). Where tested, adaption of IB viruses to growth in tissue culture has led to a rapid loss of pathogenicity for birds (von Bulow 1966a,b). There are no published reports of the propagation of Australian isolates of IB virus in tissue culture.

b) The Production of Resistance to the Australian Bronchitis Virus:

Little work has been published on the resistance induced after primary infection with IB virus with regard to such factors as the relationship between the
age of chicken, virus dose and the method of administration.

Considerable effort has been made by American workers to evaluate the immune response of vaccinated birds and their resistance to a subsequent challenge at the tracheal site. This has involved evaluating subjective estimates of infection such as tracheal rales or the amount of blood on tracheal swabs. Alternatively, the presence or actual amount of virus present in the trachea at certain times after challenge has been estimated by virus isolation and by virus titration in embryonated eggs. (Chang et al., 1957; Hitchner et al., 1964; Winterfield, 1968; Winterfield and Fadly 1971; Burke and Luginbuhl, 1972a,b).

The usual criterion of resistance when working with the Australian IB viruses experimentally has been whether or not the birds died from nephritis. Gilchrist and Sinkovic (1964, 1967) evaluated the resistance induced by an Australian IB vaccine virus given by the drinking water method at four days of age and four weeks of age. It is of interest that good resistance, as measured by mortality with nephritis, was present after the two vaccinations. The challenge experiments, using the virulent T virus (Gilchrist and Sinkovic, 1967) showed that there was only
partial resistance to the effects of the challenge virus three weeks after the first vaccination. They inferred that this was because maternal antibody prevented infection by the primary vaccination or affected the full realisation of the immune response. Fritzsche et al. (1969) in Europe using the Dutch 'H' strain of IB virus showed that birds with maternal antibody vaccinated before four weeks of age produced less neutralising antibodies than birds vaccinated at four weeks of age.

The phenomenon in Australia of outbreaks of nephritis in supposedly resistant birds after vaccination at an early age has been a problem in the field in the past (Hungerford, 1969,) and has been even more noticeable recently (Wells and Gilchrist, pers. comm.) Considering the extent of this ubiquitous problem it is most surprising that it has not been more fully investigated.

Currently in Australia, vaccination is by means of a live virus, usually passaged 25 or more times in embryonated eggs. Broiler birds are infected at four days of age and four weeks of age, or with one infection at 10-14 days of age only. These vaccinations may be followed by an additional vaccination in birds intended for laying purposes. The vaccine is more frequently administered in the drinking water, although vaccination by the 'eye drop' method is still practiced.
A number of overseas workers (Berry, 1965; Winterfield 1967; Swarbrick, et al., 1967 McMartin, 1968; McDougall, 1969; Hromatka and Raggi, 1970) have evaluated the resistance induced by beta-propiolactone inactivated IB vaccines given intramuscularly. There is some dispute as to the efficiency of the resistance induced against IB respiratory symptoms, although there may be some protection given to the reproductive tract. Inactivated vaccines have not been used in the field in Australia. Cumming (1970) reported some comparisons between inactivated vaccines and live virus vaccines. He used betapropiolactone and formalin inactivated Australian IB virus given intramuscularly. Neither of these vaccines produced a good serological response. The beta-propiolactone inactivated vaccine induced some resistance against the induction of nephritis.

c) The Relationship of the Australian Virus to Other Infectious Bronchitis Viruses:

In general Australian IB (see Cumming 1969b or Hungerford, 1969) as a disease entity is similar to other IB infections as reviewed by Cunningham (1970) Dawson (1970) and Hofstad (1972). Even the nephritis
syndrome is not necessarily exclusive to the Australian viruses as several North American and European isolates of IB virus, have been found to produce nephritis (Winterfield and Hitchner, 1962; Rinaldi et al., 1966; Mayor, 1968; Krauss and Peters, 1968). This is understandable to some extent as IB viruses are readily isolated from kidneys of infected birds (Kawakubo et al., 1961; Wells 1964; Cumming, 1967b) and kidney lesions have been found to be common in embryos infected with IB virus (Loomis et al., 1950). Although large numbers of Australian IB viruses have not been fully investigated, it would appear that the bewildering confusion of serological differences experienced overseas does not seem to be present (Cumming, 1969a; Westbury, 1970). Numerous workers have shown that the relationship between cross neutralisation and cross resistance abilities of the overseas viruses do not necessarily co-incide (Raggi and Lee, 1958, 1965; Hofstad, 1961, 1967; Hitchner et al., 1964, Winterfield et al., 1964b, and Hopkins, 1969). The small number of Australian viruses tested in this way do not seem to have these differences of resistance inducing properties (Cumming, 1967b, 1969a), although, as mentioned earlier, field outbreaks of nephritis after vaccination can occur (Hungerford, 1969).
d) Conclusion:

It would seem from the above introduction that the main approach to the disease problem associated with IB virus infections in the past has been based on the serum neutralisation relationships of the viruses involved and the ability of vaccine viruses to induce these antibodies in birds.

The approach to the problem used in this thesis has been based on the ability of virus infections to induce resistance in birds to the affects of virulent IB virus challenge and to evaluate the relationship of this resistance to antibody production. This approach has been aided by the characteristic of Australian IB viruses to cause death with nephritis under controlled experimental conditions.

The thesis falls into three parts:

1) Because of import restrictions on viruses entering Australia, foreign IB viruses were not able to be used in any of the work reported here. This meant that because an IB gel diffusion precipitation technique was not in use in Australia, the parameters of this technique had to be investigated under Australian conditions using Australian viruses. The technique
was then utilised as the primary immunological tool to detect the serological response of birds to virus infections in the following investigations.

2) Investigations were carried out into the induction of resistance against the effects of virulent virus challenge in the areas of:-
   a) the affect of the age of the bird and the presence or absence of maternal antibody at vaccination on the induction of resistance.
   b) The significance of humoral antibodies for resistance.
   c) The stimulation of protection by attenuated virus.

3) The adaption of Australian IB virus to tissue culture with a view to using this technique for virus attenuation. It was also hoped that such viruses could be titrated by a plaque count technique with the quantal benefits this latter method has for evaluating virus relationships.

   In the event, neither of these objectives was fully realised.
CHAPTER TWO

GENERAL MATERIALS AND METHODS

The experimental facilities used throughout this research work were, in the main, the same as those described by Cumming (1967b, 1969b).

Eggs: The eggs used for virus isolation, neutralisation, the production of chicks free of IB antibodies (for tissue culture and for some experiments) were produced at the Poultry Sections of the Laureldale and Kirby Rural Research Stations. The eggs were from White Leghorn hens which were monitored periodically for, and shown to be free from Salmonella pullorum and Mycoplasma gallisepticum. The Laureldale and Kirby flocks were normally free of infectious bronchitis. In the four generations of hens used for egg supply during the course of this work, only one had IB antibodies.

Chickens: The chickens normally used in the experimental work, unless otherwise indicated, were White Leghorn X Black Australorp cockerels obtained as day-old from a commercial hatchery in the Tamworth area.
The parent birds were monitored serologically and shown to be free from *S. pullorum* and *M. gallisepticum* antibodies. Although the birds were not vaccinated, IB virus antibodies were present in the dams and day-old chicks. The birds were reared in complete isolation to four weeks of age in tier brooders housed in the garages of private houses in Armidale. On no occasion were these birds found to be resistant to IB virus infection when reared in this way. The birds were moved to experimental isolation facilities at the University for actual experiments.

Experimental Facilities: Wooden isolation sheds 12' x 6' with solid floors and wire mesh covered open fronts were used as the basic units. Water was supplied from a 44 gallon drum situated outside the shed. Birds of four weeks of age or older, were housed 18" off the floor in conventional carry-on cages divided into three equal compartments. These sheds also had facilities for housing electric tier brooders for rearing chicks to four weeks of age when required.

The sheds, ten in all, were arranged in two lines of five down a paddock facing north, with at least 60'
between all sheds. The prevailing wind was from the south west.

Cold stress was produced by housing the birds in a room, approximately 14' x 16', in which both humidity and temperature could be controlled. The temperature was generally kept at 16°C (60°F). The birds in the room were housed in all metal cages, 30" x 30" x 15", fitted with feed and water troughs. The cages slid on runners into racks accommodating two tiers of four cages. Up to 35 four-week-old birds were normally placed in each cage.

The Viruses: The viruses used in the experimental work, unless otherwise stated, were the A and T isolates of IB virus (Cumming 1963, 1967) in their 28th and 11th egg passage, respectively, by the allantoic route. The A isolate, \(10^{6.8}\) egg infecting dose/50 per ml - EID\(_{50}/\)ml) a mild virus in terms of mortality from nephritis, was used mainly for the induction of resistance, and the T isolate, \(10^{7.2}\) EID\(_{50}/\)ml) a highly lethal virus in terms of mortality from nephritis, was used for challenge procedures. Birds were vaccinated or challenged individually by infraorbital instillation of one drop of a one in twenty dilution of infected allantoic fluid.

The inoculation of eggs by the allantoic and
chorio-allantoic route and the preparation of kidney specimens for virus isolation has been described by Cumming (1969b), and were adapted from Cunningham (1963).

The methods used for virus titrations followed those of Churchill (1965). Viruses were diluted in half or full log steps. In eggs, 0.1 ml of each dilution was inoculated into each of five nine-day-old embryos. Deaths occurring in the first 24 hours were disregarded as being caused by inoculation trauma. End points were determined on the basis of death, or stunting and curling of the embryos, eight days after infection. In tissue culture, 0.3 ml of each dilution was inoculated into each of three tissue culture vessels. The vessels were observed for cytopathic effect for six days. Titres were calculated by the method of Reed and Muench (1938).

Tissue culture propagated viruses were stored in sealed glass ampoules at -196°C in liquid nitrogen. Infected allantoic fluid was usually stored at -20°C in screwtopped bottles.

Immunological Techniques: Serum neutralisation tests were carried out in eggs by the constant virus method of Fontaine et al. (1963). A serum was considered to be positive if a one in four serum dilution neutralised
100 EID<sub>50</sub> of virus.

A similar method was used with tissue culture, except that 100 50% tissue culture infecting doses (100 TCID<sub>50</sub>) were used and three culture vessels per dilution.

The gel diffusion precipitin technique was basically that used by Jordan and Chubb (1962) for infectious laryngo-tracheitis. Five ml of 1% Oxoid agar No. 2, containing 8% NaCl, 1% sodium azide and veronal buffer pH 7, was pipetted onto a 7.5 x 2.5 cm microscope slide. Wells 5 mm in diameter, 2 mm between circumferences, were punched in a convenient pattern and filled with the appropriate reagents. The slide was incubated in a Petri-dish, with damp filter paper, at 37°C for at least 16 hours. The reactions were observed with an oblique light in a darkened room. For reactions to be considered positive, all sera and antigens under test had to produce precipitin lines which joined with control precipitin lines. Permanent records were made of some of these reactions by the method of Uriel and Grabar (1956) using naphthalene black as the stain on washed and dried slides, removing excess stain with a 2% acetic acid wash.

Precipitating antigens of the A or T virus were prepared from 11-day-old embryos, 48 hours after infection. Allantoic fluid was collected from batches of
live embryos, pooled and concentrated for use. Concentration of up to 40x was achieved by precipitation with an equal volume of saturated ammonium sulphate or by the addition of ammonium sulphate, 25 g/100 ml of fluid. The precipitate was collected by centrifugation and taken up in phosphate buffered saline.

Tissue Culture: Chick kidney (CK) cells were prepared by a modification of Churchill's method (1965). Kidneys taken from two or three chicks less than one week old, were finely macerated with scissors and washed twice with warm (37°C) phosphate buffered saline (PBS). The macerated kidneys were stirred with 20 ml of 0.25% trypsin in 37°C PBS for five minutes. This removed most of the red cells when the supernatent layer, without standing, was decanted. The kidney debris was then suspended in 50 ml of 0.05% trypsin in PBS and stirred at 37°C for 30 minutes. The cell suspension was centrifuged at 500 rpm for five minutes and resuspended in PBS for counting. Normally, cells were distributed in 50 ml or 100 ml medical flats at the rate of $4 \times 10^5$ cells per ml and 5 ml or 10 ml of medium per vessel respectively.

Growth medium consisted of Hank's balanced salt solution supplemented with 5% foetal calf serum, 0.15%
tryptose phosphate broth, 0.25% lactalbumen hydrolysate and appropriate levels of penicillin, streptomycin and fungizone. Maintenance medium consisted of Eagle's Basal medium made up in Earle's balanced salt solution with 2% foetal calf serum and otherwise supplemented as for growth medium. Generally, confluent monolayers resulted in 4-5 days and could be maintained for a further 7-10 days.

Coverslip cultures were produced by including one flying coverslip in a culture vessel. These were normally stained with May-Grunwald Giemsa.

Infections were initiated by removing the media from confluent monolayers of CK cells and adding 0.5ml of infected allantoic fluid directly to the cell sheet. After incubation at 37°C for 30 minutes fresh medium was added. Viruses were blind passaged by transfer of 0.5 ml of supernatent fluid to fresh cultures every four days.

Fluorescence: Fluorescent staining with coriophosphine was by the method described by Keeble and Jay (1962).

Electron Microscopy: Electron microscopy using 3% phosphotungstic acid, pH 6.4, as the negative stain,
CHAPTER 3

THE USE OF THE GEL DIFFUSION PRECIPITIN TECHNIQUE
WITH AUSTRALIAN INFECTIOUS BRONCHITIS VIRUSES.

INTRODUCTION

The gel diffusion precipitin technique has been used by several workers with European and American isolates of infectious bronchitis virus (Witter; 1962; Parisis, 1965; Woernle, 1966). Although cross neutralisation has been shown between some of these isolates and the Australian IB virus that causes nephritis (Winterfield, et al., 1964a; von Bulow, 1967; Berry and Stokes, 1968), there has been no general use of the precipitin technique with the Australian IB viruses.

This chapter reports the use of the gel diffusion precipitin technique with Australian IB viruses; the relationship between the presence of precipitating antibody and the ability to isolate IB virus from the kidneys of infected birds; and the use of technique to detect precipitating antibodies under field conditions.

a) Experimental Use of the Gel Diffusion Technique:

EXPERIMENTAL

The gel diffusion precipitin technique followed
The method of Jordan and Chubb (1962) and is described in Chapter 2.

The main method used for the preparation of precipitating antigen was by concentrating infected allantoic fluid by means of precipitation with ammonium sulphate as described in Chapter 2. This antigen is termed 'allantoic fluid antigen'. Other methods of concentrating allantoic fluid examined were freeze drying or dialysis against polyethylene glycol 20 M.

The precipitating antigen, termed 'virus precipitating antigen', was prepared by the method of Tevethia and Cunningham (1968). Briefly, after a light spin to remove cellular debris, 100 ml of infected allantoic fluid was mixed with an equal volume of phosphate buffered saline and spun at 10,000 x G for one hour. The supernatant fluid was then spun at 110,000 x G for one hour and the virus pellet resuspended in 2 ml of phosphate buffered saline. The spun virus suspension was treated with an equal volume of 'Tween'-ether (one drop of 'Tween' 80 to 20 ml of ether) for 30 minutes, followed by collection of the aqueous phase after centrifugation.

Infected chorio-allantoic membranes were processed for gel diffusion precipitating antigen by the methods of both Parisis (1965) and Woernle (1966).
Experiment 1: Twelve susceptible four-week-old cockerels were divided between two isolation pens. The birds in one pen were inoculated by infra-orbital instillation of undiluted allantoic fluid infected with the A isolate. Blood samples were collected from both groups of birds at the time of inoculation and at weekly intervals thereafter for six weeks.

This experiment was repeated, the only difference being that T virus was used instead of the A virus.

Experiment 2: Susceptible cockerels were infected at four weeks of age with the T virus and reared together under cold stress (16°C) until eight weeks of age. Ninety birds were then divided between four isolation pens. At weekly intervals ten birds (two or three from each isolation pen) were bled and killed, and their kidneys taken for attempts at virus isolation.

In one pen, ten birds were bled at weekly intervals from 4 weeks after infection to 12 weeks when the experiment was terminated.

RESULTS

The Antigen: Precipitating antigens were not detected in unconcentrated infected allantoic fluid. After concent-
ration (20-40 times) a specific line of precipitation was found (fig. 1). No differences were observed between antigens prepared from allantoic fluid infected with the A or T virus. Normal allantoic fluid, processed in the same way, was negative. The concentration method mainly used was precipitation by ammonium sulphate, although the other methods were equally successful. A non-specific line of precipitation usually appeared with most sera (normal or infected) close to the antigen well some time after the slides were removed from the incubator (fig.1).

Infected chorio-allantoic membranes were found to be unsatisfactory as a source of antigen. When tested individually, relatively few gave precipitating antigen, and this was usually weak. Pooling and concentration of disrupted infected membranes gave no better results.

Several tests were carried out during the initial preparations of the virus precipitating antigen. Precipitating antigen was not detected in the infected allantoic fluid before treatment, and neither was it detected when the final virus pellet was taken up in a fiftieth of the original volume of fluid. It was only when this latter virus suspension was treated with 'Tween'-ether to break up the virus particle that precipitating antigen was detected. Up to three lines of
precipitation were detected, one of which gave a line of identity with that produced by allantoic fluid antigen. On occasion, it had been observed that highly concentrated allantoic fluid antigen also gave more than one line of precipitation (fig. 2). 'Tween'-ether treatment of allantoic fluid antigen or infected chorio-allantoic membrane antigen did not enhance the intensity of their precipitation reactions or induce more lines of precipitation.

Serology: For normal purposes, allantoic fluid antigen producing one line of precipitation with the positive control serum was found to be satisfactory for detecting precipitating antibodies (see fig. 1).

The results of Experiment 1, showing the production of precipitating and neutralising antibody by the A virus, are given in Table 1. Precipitating and Neutralising antibodies appeared after the first week after infection. All birds tested at the third week after infection had both precipitating and neutralising antibodies which remained present until the end of the experiment at six weeks after infection. Control birds, housed under identical conditions, remained negative for both precipitating and neutralising antibodies throughout
the experimental period.

Similar results were obtained with T virus.

The results of Experiment 2, which relates the presence of precipitating antibodies and isolatable virus from four to twelve weeks after infection, are given in Table 2. Precipitating antibodies were present in all the birds when killed at the fourth, fifth and sixth week after infection. Virus was isolated from some of these birds throughout this three week period. Virus was not isolated after the sixth week of infection, but antibodies were present in the majority of birds when killed, until the end of the experiment, 12 weeks after infection.

The ten birds bled at weekly intervals from the fourth week after infection had antibodies throughout the experiment. Virus was not isolated from their kidneys at the end of the experiment, 12 weeks after infection.

DISCUSSION

Two well documented IB virus isolates, A and T, (Cumming 1963, 1967b) have been shown to produce a soluble precipitating antigen in allantoic fluid. It has been shown also that this antigen (and possibly two others) was closely associated with the virus
particle, as was shown by Tevethia and Cunningham (1962). Chickens infected with these isolates produced precipitating antibodies within the second week after inoculation, at the same time as, or slightly before, neutralising antibody appeared in the serum. This accords with the results of Woernle (1959) and Witter (1962) using European and American isolates of IB virus, but these authors reported a decline of precipitating antibody after the sixth week after infection.

It is not known why infected chorio-allantoic membranes proved unsatisfactory as a source of precipitating antigen as they have been used successfully by Woernle (1959, 1966), Witter (1962) and Hironao et al. (1970). It is of interest that Hironao et al. (1970) found some chemical differences between their single precipitating antigen derived from chorio-allantoic membranes and that of Tevethia and Cunningham (1968). However, the former authors did not make direct comparisons between their virus and the Beaudette strain of IB virus.

In the experiments reported here, the precipitating antibodies persisted in the majority of infected birds for at least 12 weeks after infection. Control birds kept under similar conditions of isolation did not produce precipitating antibodies during this time period,
and this suggests that intercurrent infection had not occurred. Although the persistence of virus in the birds could explain the continuing presence of precipitating antibodies, virus was not isolated from kidney specimens after the sixth week after infection, which confirms the results obtained by Cumming, 1969b and overseas workers (see Cunningham, 1970).

b) The Use of the Gel Diffusion Technique in Field Studies: EXPERIMENTAL

Field Survey, 1970: Four multi-aged laying farms with 10,000 or more birds, situated in the Tamworth area, were selected for the survey. Two of the farms (Q and L) did not vaccinate, whilst the other two farms (C and J) used the same commercial IB virus vaccine. At the beginning of the survey, birds from all the flocks on each farm were tested for precipitating and neutralising antibodies. In addition, the birds in at least one flock per farm were monitored for precipitating antibodies throughout the rearing period. Tests for neutralising antibodies against A virus were also carried out in many cases. Visits were made at approximately monthly intervals when blood samples were collected from at least five birds from each flock, although more samples were
RESULTS

Farm Q Unvaccinated: The site was a level piece of ground without any obstructions. In one corner of the area was an enclosed building in which each batch of chicks were reared on the floor. Open sided cage sheds were situated in the area in two rows, with at least 100 feet between sheds in the row, and approximately 50 yards between the rows.

The birds were floor reared until 8-10 weeks of age, and then transferred to a nearby carryon cage house until point of lay. The birds were then taken to a laying house.

The age structure and total number of birds on the site remained reasonably constant throughout the year.

Survey Results for Farm Q

Birds 20 weeks of age or older

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### Rearing Flocks

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<td>0/5</td>
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<td>4/4</td>
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* No positive/ No tested.
ND = Not done.

Four of five flocks over 20 weeks of age had some birds with precipitating antibodies and neutralising antibodies. Flock 3 had neither neutralising antibodies nor precipitating antibodies. Precipitating (7 of 10 birds tested) and neutralising antibodies (6 of 10 birds tested) were detected in the birds in this flock four weeks later and at the same time a 20% drop in egg production occurred.

Two flocks of birds were followed sequentially through the rearing process to point of lay. Precipitating antibodies were detected when these birds were approximately 12-14 weeks of age, just after rehousing in a rearing cage unit. The birds were eventually transferred to a laying shed.
Farm L Unvaccinated: This site had a large number of different buildings with very small spaces between them. The shedding varied from laying cage units and deep litter units housing 2000 birds, to rows of small deep litter breeding units housing only 18 birds per pen.

Eight flocks of birds over 20 weeks of age were sampled for antibody tests. Only birds in five of these flocks were positive for precipitating antibodies, however, all eight were positive for neutralising antibodies. Two of the negative precipitating antibody flocks were in their second laying year and were housed in the small deep litter breeding units. The other negative precipitating antibody flock was at point of lay in a large deep litter house.

Survey Results for Farm L

Birds 20 weeks of age or older

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<td>Agar gel test</td>
<td>3/5</td>
<td>2/5</td>
<td>4/5</td>
<td>0/5</td>
<td>2/5</td>
<td>0/5</td>
<td>0/5</td>
<td>1/5</td>
</tr>
<tr>
<td>Neutralisation</td>
<td>4/5</td>
<td>5/5</td>
<td>5/5</td>
<td>4/5</td>
<td>3/5</td>
<td>5/5</td>
<td>5/5</td>
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</tr>
</tbody>
</table>

Rearing Flocks

<table>
<thead>
<tr>
<th>Flock</th>
<th>Age (weeks)</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>15</th>
<th>20</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Agar gel test</td>
<td>0/10</td>
<td>0/10</td>
<td>0/8</td>
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<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>3/5</td>
<td>5/5</td>
</tr>
<tr>
<td>B</td>
<td>Age (weeks)</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td>15</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Agar gel test</td>
<td>0/8</td>
<td>0/5</td>
<td>0/10</td>
<td>0/10</td>
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<tr>
<td></td>
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<td>0/5</td>
<td>2/5</td>
<td>4/5</td>
<td>ND</td>
</tr>
</tbody>
</table>
The area used for the study of the rearing flocks was an area which was separated from the main farm by a road. It consisted of two large deep litter sheds and various cage sheds for holding birds. At the time of the study, there were no birds housed on the site except for the two flocks followed. No restrictions were placed on the movement of materials and personnel from other areas, indeed it was the policy to attempt to spread IB infection over the farm in this way.

Two flocks of birds were followed, each housed separately in the two deep litter sheds, but with an eight week age difference between the flocks. The birds in the two flocks did not produce precipitating antibodies before 20 weeks of age. In one of these, neutralising antibodies and precipitating antibodies were detected simultaneously, and in the other, neutralising antibodies appeared before precipitating antibodies.

Farm C Vaccinated: This site was of an oblong shape with four large laying cage houses at one narrow end, in very close proximity to each other. At the other end of the plot was a large enclosed rearing shed, capable of holding up to 2000 growing birds on deep litter. The two areas were separated by 80 yards of open, cultivated ground. Strict precautions were taken
to ensure minimal contact between the two areas.

Survey Results for Farm C.
Birds 20 Weeks of age or older.

<table>
<thead>
<tr>
<th>Flock</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar gel test</td>
<td>2/5</td>
<td>1/5</td>
<td>3/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Neutralisation</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

Rearing Flocks

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar gel test</td>
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<td>0/10</td>
<td>3/8(4/8)</td>
<td>4/10</td>
<td>6/10</td>
</tr>
<tr>
<td>Neutralisation</td>
<td>0/5</td>
<td>0/5</td>
<td>4/5(3/5)</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

NB. The birds were in the process of being transferred to the cage unit at 12 weeks of age, hence ( ) are cage unit results.

The birds in the four laying flocks had both precipitating and neutralising antibodies.

The vaccine was administered in the drinking water to birds one-day-old and again four weeks later. The young birds followed through the rearing process did not produce precipitating or neutralising antibodies before 12 weeks of age, at the time of transfer to the cage unit. Six birds taken at 8 weeks of age, placed in isolation, and challenged with T virus were resistant to the disease syndrome and produced precipitating anti-
bodies within one week of the challenge.

Farm J Vaccinated: All the houses on this site were in close proximity to each other. The birds in all four laying flocks tested had both precipitating and neutralising antibodies. Two of these flocks were being force moulted to give a prolonged laying life.

Survey Results for Farm J

Birds 20 weeks of age or older.

<table>
<thead>
<tr>
<th>Flock</th>
<th>1</th>
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<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3/5</td>
<td>3/5</td>
<td>2/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Neutralisation</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

Rearing Flock 8 weeks of age

| Agar gel test | 6/10  |
| Neutralisation | 5/5    |

The vaccine (the same commercial product as for Farm C) was given in the drinking water to birds two weeks of age. A young flock had both precipitating and neutralising antibodies when tested six weeks after vaccination.
DISCUSSION

The field survey shows the usefulness of the gel diffusion precipitin technique for detecting past infections with IB virus and for following flocks to ascertain the approximate time of exposure to the virus. Unfortunately, because of the persistence of precipitating antibody in infected birds, we were unable to detect secondary infection by this technique as had been done by Woernle (1966).

In the initial screening, only birds from five of the 21 laying flocks tested on the four farms did not have IB precipitating antibodies, even though not all the birds tested in any one flock had these antibodies. All the flocks followed through the rearing process had some birds that eventually produced precipitating antibodies. These were detected at a similar time to neutralising antibodies. The precipitating antibodies were detected in some birds each time a flock was sampled after it became positive. No overt disease syndrome developed on the two unvaccinated farms, suggesting that the viruses infecting these flocks was very mild.

The testing of birds in one or two flocks for IB virus antibodies throughout the rearing period gave some insight into the relationship between management procedures on the farms and the time of infection with IB
virus. On farm Q, the young chicks were always brooded in one house, and from the results, it would seem that infection occurred on rehousing in a cage unit at about 12 weeks of age. The birds were finally housed in laying units at point of lay. The bird population and age group structure on this farm remained fairly constant. As was shown, however, an occasional batch of birds was able to go through this cyclic regime without IB infection.

Farm L had a regime of population and depopulation at certain times of the year. Birds were also moved frequently from shed to shed. The survey was carried out at the beginning of the repopulation cycle. Under this regime one might expect a greater variability in the time at which the infection occurred. In the two batches of birds that were followed, the infection occurred near to sexual maturity and could have affected the initial egg production. It would seem that a depopulation regime could lead to serious problems from IB infection, by breaking the cycle of natural infection or by infection during the laying period, which could affect egg production. Farm Q said that they had no problems with clinical IB, whereas farm L said that they had problems occasionally with the disease during rearing and when birds were in lay.
The two vaccinated farms differed also in their management regimes. Farm C reared birds in good isolation until 12-14 weeks of age, before rehousing in the final cage unit. Antibodies were detected at the time of this move in both the rearing unit and the cage unit. At such a time the birds were more at risk to secondary infection, and this may have produced the antibodies. Six birds challenged with T virus under our isolation conditions before this time were resistant and produced precipitating antibodies within one week of challenge. It is not known why antibodies were not produced earlier with the vaccine schedule used. However, Raggi and Lee (1965 have shown that detectable antibodies are not essential for resistance to secondary challenge (see also Chapter 5).

Farm J reared their birds in much closer proximity to the other flocks on the premises so it was not certain whether the antibodies were the result of vaccination or a secondary infection, or both. Neither of these two farms had problems with clinical IB.

The field survey confirmed the usefulness of the gel diffusion technique for evaluating the previous exposure of a flock to IB virus. The absence of precipitating antibodies is not, however, an indication that the birds have not experienced infection with IB virus. The detection of precipitating antibody can be used only as an
initial screening device; all flocks of birds found to be negative by this method should be tested for neutralising antibodies. However, large numbers of samples can be screened by the gel diffusion technique, in a simple rapid manner, leaving fewer samples to be tested by the more sensitive but cumbersome neutralisation method using embryo malformation as the end point.

SUMMARY

The agar gel diffusion precipitin technique was evaluated using a soluble precipitating antigen from allantoic fluid infected with an Australian infectious bronchitis virus under Australian conditions.

The appearance of precipitating and neutralising antibodies under experimental and field conditions was described. The precipitating antibody appeared to persist for at least 12 weeks after infection, although no relationship could be found between its presence and the isolation of virus from kidneys of infected chickens. The agar gel precipitin technique would appear to be useful as an initial test for screening large numbers of samples to determine whether flocks of birds had experienced IB virus infections.
TABLE 1
Detection of Precipitating and Neutralising Antibodies
to Infectious Bronchitis Virus by Cockerels Inoculated
with Isolate A at 4 Weeks of Age.

<table>
<thead>
<tr>
<th>Cockerel No.</th>
<th>Presence of Precipitating and Neutralising Antibodies at 0 to 6 weeks after Inoculation.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>4</td>
<td>-</td>
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<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: - = negative for precipitating antibody.
+ = precipitating antibody present.
N = neutralising antibody present.
Control cockerels were negative for both antibodies throughout the experiment.
Precipitating Serum Antibodies and Isolation of Infectious Bronchitis Virus from Kidney Samples from 10 Cockerels Killed at 4-12 Weeks After Infection with Isolate T

<table>
<thead>
<tr>
<th>Weeks after Infection</th>
<th>Precipitating Antibody</th>
<th>Isolate of Virus from Kidneys</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>10/10*</td>
<td>7/10</td>
</tr>
<tr>
<td>5</td>
<td>10/10</td>
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<tr>
<td>6</td>
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<td>0/10</td>
</tr>
<tr>
<td>12</td>
<td>10/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

*Numerator is number of cockerels showing antibody or yielding IB virus.
Denominator is number of cockerels examined.
specific reactions

non-specific reactions
Figure 1: This is a typical stained gel diffusion slide showing a test for infectious bronchitis precipitating antibodies in unknown sera. Some sera from laying birds give lipid deposits which may obscure the reactions if left for longer than 24 hours to diffuse.

The two outer rows of wells contained the unknown sera under test. The centre row of wells contained, alternatively, a known positive infectious bronchitis anti-serum and a known positive infectious bronchitis allantoic fluid antigen. The inner line of precipitation, closest to the antigen well, was a non-specific reaction seen with some normal sera and normal allantoic fluids. The outer, specific precipitation line, can only join with precipitin lines produced by sera with infectious bronchitis precipitating antibodies.

The last well of the bottom row did not contain any reagents. The sera in the other wells of the outer rows were, in varying degrees, positive for infectious bronchitis precipitating antibodies.

Figure 2: This is a stained gel diffusion slide showing the complexity of the infectious bronchitis virus precipitation reaction when highly concentrated infected allantoic fluid and virus derived antigens were reacted with hyperimmune anti-infectious bronchitis virus serum.

The wells contained, in a, hyperimmune serum, b, allantoic fluid derived antigen, and c, virus derived antigen.

In some cases at least three precipitation lines could be seen, as well as the non-specific line of precipitation.
non-specific reactions

specific reactions

specific reactions
CHAPTER 4

THE EFFECTIVENESS OF VACCINATING BIRDS BEFORE TWO WEEKS OF AGE AGAINST THE AUSTRALIAN INFECTIONOUS BRONCHITIS VIRUS

INTRODUCTION

Apart from the work of Raggi and Lee (1953, 1965), there has been little work published on the effectiveness and duration of resistance to infection by avian infectious bronchitis virus after vaccination with live virus during the first month of life.

Following isolation of IB virus from Australian birds by Cumming (1962, 1963), Gilchrist and Sinkovic (1964) described a vaccine which showed good protection against the disease under laboratory and field conditions (Gilchrist and Sinkovic, 1967). Since that time, vaccination against IB infection with live, egg adapted, virus has become a standard preventative measure against the disease in Australia, (Cumming, 1969a). Apart from the field trials of Smith et al., (1971) there have been no other publications on the induction of resistance with Australian IB vaccines.
This chapter attempts to assess the effectiveness of vaccination before twenty eight days of age against challenge with a virulent nephritis inducing IB virus and the relationship of any response with the presence or absence of precipitating antibody.

EXPERIMENTAL

The experimental chickens with maternal antibody to IB were commercially available White Leghorn X Black Australorp cockerels obtained at day-old from a local hatchery. The chickens of mixed sex, without maternal antibody, used in experiment 3 were obtained by hatching eggs derived from birds at the University farm. The parent birds were free of IB neutralising antibody and had shown no symptoms of IB. (See Chapter 2). The birds were reared in tier brooders in the isolation sheds until three weeks of age and then transferred to carry-on cages in the same shed until used.

Vaccination and challenge of birds was carried out by instillation of 1/20 dilution of infected allantoic fluid into the infra orbital sinus (see Chapter 2)

Experiment 1: Chicks with maternal antibody were reared in isolation until vaccinated at 1, 4, 7, 10, 14, 21 and 28 days of age with the A virus. Each vaccinated
age group was held in a separate isolation shed and supplied with additional heat and electrolyte replacers to prevent losses from nephritis (Cumming, 1967; Cumming and Heath 1969). At 2, 4, 8, and 16 weeks after vaccination, approximately 50 vaccinated birds were taken from each age group, challenged with the T virus, and held in a cold room at 13°C for three weeks. Ten unvaccinated birds reared under identical conditions acted as a control for each challenge.

All birds that died were autopsied. Susceptibility to challenge with T virus was determined by death from kidney failure (Cumming, 1963, 1967b).

The antibody status of the birds was monitored by sample bleedings of five birds taken at vaccination and challenge and tested for precipitating antibodies.

Experiment 2: Essentially, Experiment 1 was repeated, but the chickens were not vaccinated after 14 days of age and were not challenged beyond four weeks after vaccination. The cold room was held at 16°C, a slightly higher temperature than used in Experiment 1 because of technical difficulties with the refrigeration system.

Experiment 3: The same experimental regimen as Experiment 2 was used, except that the chicks were of mixed
sex and had no maternal antibody. Unfortunately not enough birds were available to challenge all the vaccinated groups at four weeks after vaccination. Sexing was carried out at the end of the experiment or at post-mortem.

RESULTS.

The results of all three experiments are given in tables 3, 4 and 5. To facilitate comparison, part of the results of Experiment 1, contained in table 3, are repeated in tables 4 and 5.

Experiment 1 (table 3). A total of 200 birds were vaccinated with the A virus at each age group. A few birds in each group died during the first week of rearing because of overcrowding, hence, less than 50 birds were challenged 16 weeks after vaccination. No other birds died in any group before they were challenged with the T virus under cold stress.

Control, unvaccinated birds, remained susceptible to challenge with T virus, under cold stress, throughout the experiment. Mortalities with nephritis ranged from two out of ten birds to eight out of ten birds and occurred between 6 and 14 days after challenge. Any vaccinated birds that died after T virus challenge also had nephritis and died between 6 and 14 days after
challenge.

No birds died with nephritis in any vaccinated group of birds when challenged with T virus at 8 or 16 weeks after vaccination. Groups of birds vaccinated at 14, 21 and 28 days of age were resistant to T virus challenge when tested at two and four weeks after vaccination or later, whereas, groups of birds vaccinated at 4, 7 and 10 days of age were only resistant at four weeks after vaccination or later.

The group of birds vaccinated at one day of age had one of the fifty birds die with nephritis after they were challenged with T virus four weeks after vaccination. Groups of birds vaccinated at 1, 4, 7 and 10 days of age had mortalities with nephritis ranging from 2-20% after challenge with the T virus at 2 weeks after vaccination. Unvaccinated birds challenged with T virus at the same time had mortalities with nephritis ranging from 20-70%.

Maternal antibodies were detected by the gel diffusion test in samples of unvaccinated birds tested at 1, 4 and 7 days of age. 'Active' precipitating antibodies were found in birds vaccinated at these ages at four weeks after vaccination or later, but not at two weeks after vaccination. Unvaccinated birds sampled at ten days of age, or older, had no 'passive'
precipitating antibodies, but 'active' precipitating antibodies were detected in vaccinated samples of these birds two weeks after vaccination or later. The presence of 'active' precipitating antibodies in vaccinated groups at challenge with T virus coincided with a marked drop in mortality after the challenge.

Experiment 2 (tables 4 & 5). No birds died before the birds were challenged with T virus in the cold room. Essentially the results were similar to Experiment 1. Some birds died with nephritis (6-12%) in those groups of birds vaccinated at 1, 4, 7 and 10 days of age after challenge with the T virus two weeks after vaccination. The birds died between 6 and 15 days after the challenge. No birds died when samples of birds vaccinated at these ages were challenged four weeks after vaccination. Birds vaccinated at 14 days of age were resistant to T virus challenge at 2 or 4 weeks after vaccination. Control, unvaccinated groups of birds, challenged with T virus at the same time as the various vaccinated groups of birds, all had some mortality (20-70%) with nephritis from six days after the challenge.

Maternal antibody was detected by the gel diffusion precipitin test in samples of unvaccinated birds until
10 days of age, three days later than Experiment 1. In a similar manner to Experiment 1, no 'active' precipitating antibodies were detected in groups of birds vaccinated with maternal antibody (ie. 10 days of age or earlier) until four weeks after vaccination. Samples of birds tested at 14 days of age had no detectable precipitating antibodies. Precipitating antibodies were detected in groups of these vaccinated birds at 2 and 4 weeks after vaccination. The presence of 'active' precipitating antibodies in vaccinated groups at challenge with T virus co-incided with a marked drop in mortality after the challenge.

Experiment 3 (tables 4 & 5:) The results of this experiment, especially when the cockerels alone are considered, follow closely the results obtained for Experiments 1 and 2. Control unvaccinated birds were susceptible to T virus challenge throughout the experiment. Challenge with T virus at two weeks after vaccination caused some mortality with nephritis in groups of birds vaccinated at 1, 4 and 7 days of age, but not in birds vaccinated at 10 days of age. One of thirteen cockerels vaccinated at one day of age died with nephritis when they were challenged with T virus four weeks after vaccination, whereas, twenty
Cockerels vaccinated at 4 days of age were resistant to T virus challenge at this time.

No maternal precipitating antibodies were detected before vaccination in each of the age groups of birds used. Precipitating antibodies were detected by four weeks after vaccination in samples of birds vaccinated at 1, 4 and 7 days of age, and at two weeks after vaccination in a sample from birds vaccinated at 10 days of age.

The mortality (7.6-24%) with nephritis after T virus challenge was not significantly different to Experiments 1 and 2 despite the lack of maternal antibody. Similarly, the presence of 'active' precipitating antibody and a drop in mortality occurred in a similar manner to Experiments 1 and 2. The results for female birds were of a similar pattern to males, but mortality was lower.

DISCUSSION

The experiments reported here suggest that vaccination against IB virus infection before 10 days of age does not produce full resistance to challenge with a virulent virus by 14 days after vaccination. This period coincides with an inability to produce precipitating antibody. When precipitating antibodies were produced by the time of the challenge
the chicks were generally found to be resistant. 

The duration of the resistance was shown in Experiment 1 to last for at least sixteen weeks after vaccination, and it was found, as suggested previously (Chapter 3, Chubb and Cumming, 1971), that precipitating antibodies were still present.' This resistance could not be due to an age effect as the control groups of birds had similar mortalities after challenge at all ages tested. Nevertheless, although mortality can be experienced from an early challenge with IB virus in birds vaccinated before 14 days of age, this does not reach as high a level as non-vaccinated birds, suggesting a partial immunity.

Although female birds are generally more resistant than cockerels to the nephritis syndrome (Cumming, 1967b), the results for females in Experiment 3 follow the same general pattern as for cockerels.

It might be thought that some mortalities after challenge of vaccinated birds under cold stress could be caused by the vaccine virus rather than by the challenge virus. Cumming (unpublished) however, has shown that the time sequence of mortality in infected birds is not altered even if the birds are cold stressed later than from the time of infection. As such, any mortality attributable to the vaccine
should have occurred well before six days after vaccinated birds were challenged with T virus when the mortalities occurred in the experiments reported here.

Several Australian workers have suggested that a breakdown of immunity occurs in chickens vaccinated at an early age because of losses from nephritis in broiler flocks several weeks after vaccination (Gilchrist and Sinkovic, 1967; Hungerford, 1969; Wells and Gilchrist, pers. comm., 1973). Using Australian isolates of IB virus, Gilchrist and Sinkovic (1967) noted mortality from a challenge with T virus three weeks after vaccination. The authors suggest that this outbreak of nephritis was due to maternal antibody interfering with the original vaccination of the birds at four days of age. Comparison of Experiment 3 with Experiments 1 and 2 showed that this mortality from early challenge with virulent virus was not adversely affected by the presence of passive antibody from the dam. Maternal antibody in these experiments, therefore, did not affect the induction of resistance, or of precipitating antibody.

Raggi and Lee (1958, 1965) showed that passive antibody from the dam did not affect resistance to challenge in the long term as measured by tracheal
rales, when American isolates of IB virus were tested. They did show a significant difference in resistance when birds vaccinated at six days of age were compared with birds vaccinated at two months of age. This difference was not observed when less virus was used for vaccination. The results here support their findings in that maternal antibody did not adversely affect resistance. Differences in resistance in the long term were not found in birds vaccinated over the first 14 days of life, but there were short term differences when birds were challenged two weeks after vaccination.

Fritzsche et al. (1969) showed that the presence of maternal antibodies at vaccination could reduce subsequent antibody responses to vaccination and challenge with the homologous H120 and H52 strains of IB virus. No challenge work was carried out with virulent viruses to see whether this depressed antibody response correlated with differences in the ability of birds to resist the affects of IB virus infections.

Raggi and Lee (1965) found no correlation between antibody response to vaccination and clinical resistance to the challenge virus. In Chapter 5 it will be shown that suppression of the antibody response to IB
vaccination with cyclophosphamide did not affect resistance. The present work shows that resistance in early life co-incides with the production of antibody after vaccination. If this antibody is not directly related to resistance, then it would seem that its production merely co-incides with a change in the immunological competence of the bird.

Based on experience from the United States, Gilchrist and Sinkovic (1964) suggest that broilers be vaccinated at four days and four weeks of age. Using this regime, Gilchrist and Sinkovic (1967) recorded some mortality on challenge with T virus at three weeks of age but not at five weeks of age, a week after the second vaccination. These observations support the results given in this chapter.

Smith et al. (1971) studied the serological responses to vaccination at four weeks of age and 16 weeks of age in field trials with Australian vaccine on laying stock. Although no adverse affect was seen after vaccination, no specific challenge with virulent virus was given to test the efficiency of the vaccine schedule.

The results presented in this chapter support the results of Gilchrist and Sinkovic (1967) by showing that birds vaccinated at an early age do not have
full resistance to IB virus infection by 3 weeks of age. However, the results go further, and show that nevertheless, full resistance is generally reached by four weeks after vaccination in birds vaccinated between 4 and 10 days of age, and by two weeks after vaccination in birds vaccinated at 14 days of age. A second vaccination is not essential for the full expression of this resistance response.

It is suggested, from the experiments reported here, that a single vaccination at 10-14 days of age should produce resistance to re-infection with IB virus for a fairly long period of time, (16 weeks or more) sufficient at least for broilers. Further, whilst the chicks may not be fully resistant for two weeks if vaccination is carried out before 10-14 days of age, there does not seem to be a need to re-vaccinate at four weeks of age. As an additional insurance against subsequent egg loss, a second vaccination is recommended for birds intended for laying purposes, although this needs further investigation as the duration of immunity to IB is not well documented, particularly under field conditions.

SUMMARY

Vaccination with an Australian avian infectious
bronchitis virus between one and ten days of age did not result in effective resistance to a challenge with a virulent virus for at least four weeks after vaccination. The presence or absence of maternal antibody did not affect this finding. Chicks vaccinated at 14 days of age, or later, were fully resistant 14 days after vaccination. Whatever the time of vaccination, all birds vaccinated were resistant to challenge four weeks after vaccination. This resistance seemed to co-incide with an ability to produce precipitating antibody by the time of challenge.
TABLE 3

Experiment 1. The Antibody Response to Vaccination of Birds up to four weeks of age with A Virus and their Resistance to Challenge with T. Virus.

<table>
<thead>
<tr>
<th>Age at vaccination (days)</th>
<th>0 M</th>
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<th>4</th>
<th>8</th>
<th>16</th>
</tr>
</thead>
<tbody>
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<td>V</td>
<td>C</td>
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<tr>
<td>28</td>
<td>0/5</td>
<td>5/5</td>
<td>0/50</td>
<td>3/10</td>
<td>5/5</td>
</tr>
</tbody>
</table>

M - No. of birds with maternal antibody (precipitins) at vaccination/No. of birds tested
A - No. of birds with precipitating antibody/No. of birds tested.
V - Mortality with nephritis after challenge/No. of vaccinated birds challenged.
C - Mortality with nephritis after challenge/No. of unvaccinated birds challenged.
TABLE 4
Mortality After Challenge with T Virus at
Two or Four Weeks After Vaccination

<table>
<thead>
<tr>
<th>Age at Vaccination (days)</th>
<th>Chickens with maternal antibody</th>
<th>Chickens without maternal antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*3/50 2/10</td>
<td>1/50 7/10</td>
</tr>
<tr>
<td>4</td>
<td>8/50 3/10</td>
<td>0/50 5/10</td>
</tr>
<tr>
<td>7</td>
<td>10/50 7/10</td>
<td>0/50 3/10</td>
</tr>
<tr>
<td>10</td>
<td>1/50 4/10</td>
<td>0/50 3/10</td>
</tr>
<tr>
<td>14</td>
<td>0/50 8/10</td>
<td>0/50 4/10</td>
</tr>
</tbody>
</table>

*Mortality/total No. of birds.
+ ND - Not done because of lack of birds.
### TABLE 5
The Screening of Birds for Precipitation Antibody at Vaccination and at Challenge with T Virus Two and Four Weeks Later.

<table>
<thead>
<tr>
<th>Age at Vaccination (days)</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At Vacc. 2 wks. 4 wks.</td>
<td></td>
<td>At Vacc. 2 wks. 4 wks.</td>
<td></td>
<td>At Vacc. 2 wks. 4 wks.</td>
</tr>
<tr>
<td>1</td>
<td>*3/5 0/5 4/5</td>
<td></td>
<td>5/5 0/5 5/5</td>
<td></td>
<td>0/5 0/5 4/5</td>
</tr>
<tr>
<td>4</td>
<td>3/4 0/5 5/5</td>
<td></td>
<td>5/5 0/5 5/5</td>
<td></td>
<td>0/5 0/5 5/5</td>
</tr>
<tr>
<td>7</td>
<td>2/5 0/5 5/5</td>
<td></td>
<td>1/5 0/5 5/5</td>
<td></td>
<td>0/5 0/5 ND+</td>
</tr>
<tr>
<td>10</td>
<td>0/5 1/5 5/5</td>
<td></td>
<td>1/5 0/5 5/5</td>
<td></td>
<td>0/5 1/5 ND</td>
</tr>
<tr>
<td>14</td>
<td>0/5 4/5 5/5</td>
<td></td>
<td>0/5 1/5 5/5</td>
<td></td>
<td>ND ND ND</td>
</tr>
</tbody>
</table>

Control birds had no detectable anti-body at challenge.

* No. of birds with precipitating antibody/No. of birds tested.

+ ND - Not Done because of lack of birds.