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Fowl-Pox Vaccination in South Africa with Egg-Propagated Fowl and Pigeon-Pox Viruses.

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Fowl-pox is widespread in the Union of South Africa. The incidence of the disease follows roughly the rise and fall in density of the mosquito population and it appears that in this country fowl-pox is transmitted almost exclusively by mosquitoes. Outbreaks occur in spring and early summer, but it is in the autumn, i.e. in March, April and May that the disease is most prevalent.

Apart from known vectors of the disease which are found in South Africa, it has been shown by Coles (1947) that *Culex theileri* is a transmitter and the opportunity is taken of recording this observation.

Lesions are usually confined to those parts of the body which are exposed to attack by mosquitoes. In fowls it is the comb and wattles which are affected and the disease may spread to the eyes. Mouth lesions are found in rather less than 10 per cent. of cases. Occasionally lesions occur on the skin of the body. Leg lesions are common in turkeys but are rarely seen in chicks.

In 1923 Mitchell (unpublished work cited by Canham 1932), working at the Allerton Laboratory in Natal, prepared a vaccine from fowl-pox virus grown on the combs of cockerels. This vaccine was used on a small scale with reasonably good results until 1932 when following the work of de Blieck (1927) and others, it was replaced by Canham (1932) with one made from pigeon-pox virus grown on pigeons.

The pigeon-pox vaccine has been in use about 15 years and has been of considerable value in combating the disease. It has been found in practice, however, that the immunity conferred is limited and that after 3 months it has apparently decreased considerably. Similar observations have been reported by other workers. Glover (1939) stated that the duration and solidity of the immunity conferred by pigeon-pox virus may vary considerably.

In many parts of South Africa vaccination must be carried out early in summer. In some of the warmer areas the disease may be so severe even in spring that baby chicks and poults must be vaccinated soon after they are hatched and protected from mosquitoes until immunity has developed. Thus by the time the incidence of the disease has reached its peak in the autumn, birds that were vaccinated with pigeon-pox virus have very little immunity and many become infected. These birds usually show greatly modified reactions and are seldom visibly sick, but they are thrown out of production and the financial loss to the farmer may be severe.

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Vaccine produced from pigeons is costly and the method of production is undoubtedly brutal. The pigeons used are obtained from all parts of the Union and it is known that many are infected with psittacosis (Coles, 1940 and Canham 1947). These birds are a potential danger to those who have to handle them. In spite of the widespread occurrence of the disease, however, no human cases have been reported in the Union. Salmonellosis is perhaps a greater danger since *Salmonella typhimurium* has frequently been isolated from pigeons (Henning and Haig, 1939).

Fowl-pox virus produces a solid and lasting immunity (Pyle 1929), and vaccines made with virus grown on the combs of cockerels have been widely used. Such vaccines may, however, be too virulent for general use and there are numerous obvious objections to the method of production (Canham 1932 and Glover 1939).

The effect of fowl-pox vaccine on birds was studied by Lubbehusen and Beach (1938). They found that there was a marked systemic reaction in birds vaccinated over the age of 12 weeks and this resulted in a temporary inhibition of growth. In younger birds the reaction decreased progressively as the age of the birds rose from 1 day to 6 weeks, and in an earlier publication (Lubbehusen, Beach and Busie, 1936) it is stated that this reaction does not occur when birds, eight weeks old, are vaccinated. To substantiate their observations they cite Bice (1939) who found the optimum age for vaccination to be from 4 to 12 weeks.

It was with the virus of fowl-pox that Woodruff and Goodpasture (1931) did their classical work on the use of the developing egg as a medium for virus cultivation. It has since been shown by Beaudette and Hudson (1938) that pigeon-pox virus can be grown readily in eggs by serial passage. Both pigeon-and fowl-pox viruses grown in this way have been used to prepare vaccines against fowl-pox (Brandy 1936, and Beaudette and Hudson 1938). Such products are bacteria free and devoid of the objections raised when pigeons or cockerels are used.

Serial passage of pigeon virus in eggs resulted in no clearcut improvement in immunising ability but it did seem to increase avidity for fowls (Glover 1939 and personal observations). De Blieck (1947), however, stated that the keeping quality of pigeon virus is poorer when grown in eggs than on pigeons.

Passage of fowl virus in eggs resulted in slight attenuation for fowls (Glover 1939), and the egg-adapted virus showed no tendency to spread from the inoculation site.

For the reasons outlined above it was decided to produce vaccines for use in the Union of South Africa from both pigeon-and fowl-pox viruses grown in developing eggs, and to abandon the vaccine made from pigeons, the fowl-pox vaccine for general use on birds from 4 to 12 weeks of age, and the pigeon-pox vaccine for use where birds of any other age were to be vaccinated.

Methods and Materials.

Eggs from a pullorum-free flock of white leghorns kept at the Institution were delivered to the laboratory after preliminary incubation in a Jamesway forced draught incubator at 100° F.

Usually the embryos were 11 days old but occasionally younger or older embryos were used.

All injections were made onto the chorioallantoic membranes by Alexander's (1938) modification of Burnet's technique. The method is rapid and eminently suited to such work as the titration of virus activity and the mass production of virus for vaccine where large numbers of eggs must be handled.

When large numbers of eggs are handled it is essential that the manipulation of the chorioallantoic membrane be done soon after the groove in the shell is made, otherwise some drying of the membrane occurs which causes it to adhere firmly to the shell membrane.

An egg-adapted strain of fowl-pox in its 88th egg generation was obtained from Dr. F. R. Beaudette of the New Jersey Agricultural Experiment Station to whom we take this opportunity of expressing our thanks. The strain was passed rapidly through a further 8 egg generations and was then stored as disintegrated infected membranes in a refrigerator at 4° C. At approximately three-monthly intervals the strain was rejuvenated by a single passage through eggs.

Two strains of pigeon-pox virus were obtained from Dr. A. S. Canham of the Allerton Laboratory, Pietermaritzburg, Natal. One, strain D, was the strain used for the production of vaccine from pigeons in that Laboratory. The other, strain D2, was collected from a wild dove. After these strains had been isolated in eggs, they were stored in the same way as the fowl strain.

Growth of virus in eggs was judged by the macroscopic appearance of infected chorioallantoic membranes and in some cases smears from these membranes were made and stained with Victoria Blue by Kaiser's (1938) modification of Hertzberg's method. The method was found excellent for membranes infected with fowl-pox but was unsatisfactory for those infected with pigeon-pox.

Except where special mention is made titrations of virus activity were made on eleven day embryos. Serial ten-fold dilutions of the material to be tested were made in ordinary broth and six eggs were used for each dilution. The dose for each egg was 0·15 c.c. After injection the eggs were re-incubated at 35° C. and the membranes were examined for lesions on the fourth day after injection in pigeon-pox titrations, and on the sixth day in fowl-pox titrations.

The end-point of a titration was taken as the dilution which produced definite lesions in at least half the eggs injected.

Growth in Eggs.

A. *Fowl-pox Virus.*

The Influence of the temperature of incubation

To study the influence of the temperature of incubation on the growth of fowl-pox virus in eggs, serial decimal dilutions of fluid obtained by angle-head centrifugation of freshly harvested membranes disintegrated in Alexander's (1947) mincer tubes were prepared.

Four groups of six eggs were injected with each dilution. One group was incubated at 32° C., one at 33·5° C., one at 35° C. and the remaining group at 37°C. Seven days later the surviving eggs were opened and the chorioallantoic membranes examined.

The results of this experiment are given in table 1.

TABLE 1.—*The influence of the temperature of incubation on the growth of fowl-pox virus in eggs.*

Dilution of Virus.	Temperature of Incubation.			
	32° C.	33·5° C.	35° C.	37° C.
10^{-2}	+++	++++	++++	++++
10^{-3}	++±	+++	++±	++++
10^{-4}	++	+++	+++	++++
10^{-5}	+	++	++±	+++
10^{-6}	—	—	+	++

Result.—From table 1 it is seen that the lesions were most extensive when the eggs were incubated at 37° C. The influence of temperature on growth was most noticeable when dilute suspensions were used as inoculum. Thus as 32° C. the 10^{-6} suspension produced no visible lesions whereas in those eggs incubated at 37° this suspension caused well-marked lesions.

Early deaths, however, were more numerous at 37° C. than at the lower temperatures.

Comment.—This experiment showed that the lesions produced by fowl-pox virus were greatest when the injected eggs were held at 37° C. than when lower temperatures of incubation were used. The virus activity of the lesions in the eggs held at the different temperatures was not determined but it was assumed that the greater the lesion the greater the total amount of virus in the membrane would be.

As multiplication at 35° C. was apparently only slightly less than at 37° C. and because there were fewer non-specific deaths at this temperature of incubation, all subsequent work was done at 35° C. Numerous titrations of virus grown at 35° C. gave values between 10^{-5} and 10^{-7}.

Length of time allowed for virus multiplication.

Serial decimal dilutions of supernatant fluid from freshly harvested and disintegrated membranes were made in broth and were used to infect four groups of eggs. These eggs were incubated at 35° C. On the third day after injection the eggs of one group were opened and the membranes examined. Similarly on the 4th, 5th and 6th days the other groups of eggs were examined.

The results of this experiment are set out in table 2.

TABLE 2.—*The influence of the time allowed on growth of fowl-pox virus in eggs.*

Dilution of Virus.	Number of Days of Incubation at 35° C.			
	3.	4.	5.	6.
10^{-2}	+++	++++	++++	++++
10^{-3}	+++	++++	++++	++++
10^{-4}	++±	+++	++++	++++
10^{-5}	++	+++	+++	+++
10^{-6}	±	±	+	+
10^{-7}	—	±	—	±

Result.—From table 2 it is seen that by the third day of incubation the lesions were well developed and the end-point could be determined. When further time was allowed for growth, the lesions, especially in those eggs that received large doses of virus, extended considerably.

In a second experiment a group of 18 eggs received a 10^{-2} dilution of the supernatant fluid from freshly harvested membranes. On the third day after incubation at 35° C. 3 eggs were selected at random and the entire chorioallantoic membranes were harvested and titrated in eggs. Again on the 5th and 7th days other eggs were taken and the virus activity of the entire membranes measured.

The end points obtained from these titrations were 10^{-4} from the membranes harvested on the 3rd day of incubation, 10^{-5} from those harvested on the 4th day and 10^{-6} from those harvested on the 7th day.

Comment.—In these experiments the lesions produced by fowl-pox virus in eggs increased in extent with prolonged incubation and virus activity in the membranes showed a corresponding increase. However, on the seventh day of incubation a number of dead embryos were found. It was also evident that more prolific growth was obtained when inocula rich in virus were used to infect the eggs; when the seed material was highly diluted the lesions produced were small and these showed little tendency to increase in size with prolonged incubation.

Therefore, when large amounts of virus were required for vaccine production a 10^{-2} dilution of freshly harvested material was used to infect the eggs and the membranes were collected on the 6th day of incubation. In this way consistently good yields were obtained.

Distribution of the virus in infected Eggs.

A 10^{-2} dilution of stock suspension was used to infect the chorioallantoic membranes of six eggs preincubated 11 days. After injection they were incubated at 35° C.

Five days later the embryos, chorioallantoic membranes and the yolk-sacs were harvested separately and the virus activity determined by titration in eggs.

The results of these titrations are given in table 3.

TABLE 3.—*The distribution of fowl-pox virus in infected eggs.*

Dilution.	Yolk-sacs.	Embryos.	C.A. Membranes.
10^{-3}	++	+++	++++
10^{-4}	±	+	++++
10^{-5}	—	—	+++
10^{-6}	—	—	+
10^{-7}	—	—	±

Result.—From table 3 it is seen that the embryos and yolk-sacs contain appreciable amounts of virus but the concentration in the membranes is considerably higher.

Comment.—Thorning, Graham and Levine (1934) found that the virus in infected eggs was not confined to the chorioallantoic membranes and they recommended the use of the whole egg for vaccine production. A small batch of vaccine

was prepared in this laboratory in which the entire contents of infected eggs were suspended in glycerine-broth medium. The mixture contained 45 per cent. glycerine and 25 per cent. egg material, and was issued to a number of poultry farmers. Takes were good but the farmers were worried by the presence of small feathers in the vaccine and considered it contaminated. As the total virus content of parts of the egg other than the chorioallantoic membrane is relatively small, infected membranes alone are now used for vaccine production.

Influence of the age of the embryo on the multiplication of virus.

A bottle of fowl-pox vaccine containing 15 per cent. disintegrated membrane suspended in glycerine-broth (see later) was selected and serial decimal dilutions were made in broth. The membranes of three groups of eggs were infected with each dilution. The eggs in the first group contained 8 day old embryos, those in the second 10 day old embryos and those in the 3rd, 12 day old embryos. The eggs were then incubated at 35° C. and their membranes were examined 5 days later. The results of this experiment are shown in table 4.

TABLE 4.—*Multiplication of fowl-pox virus in embryos of different ages.*

Dilution of Inoculum.	Age of Embryos when Injected.		
	8 Days.	10 Days.	12 Days.
10^{-2}	++++	+++++	+++++
10^{-3}	+++	++++	++++
10^{-4}	+	+++	+++
10^{-5}	+	++	+++
10^{-6}	+	+	++

Result.—From table 4 it is seen that the end point of the titration in the 3 groups of eggs was approximately the same. The lesions were, however, sonsiderably more extensive in the older eggs. In the younger eggs the lesions were markedly oedematous and there appeared to be very little cellular proliferation.

From those eggs of the different groups injected with the 10^{-2} dilution of the virus suspension (table 4) the chorioallantoic membranes were harvested separately and the virus titre determined in 11 day embryos. The results of this experiment are shown in table 5.

TABLE 5.—*The virus activity of chorioallantoic membranes of embryos* 8, 10 *and* 12 *days old when injected.*

Dilution of Inoculum.	Membranes from		
	8 Day Embryos.	10 Day Embryos.	12 Day Embryos.
10^{-3}	++++	+++	++++
10^{-4}	+++	+++	—
10^{-5}	++	++	+++
10^{-6}	+	+	+

Result.—From table 5 it is seen that there is no apparent difference in the virus titre of membranes taken from eggs that were injected when 8, 10, or 12 days of age. There was, however, a considerable increase in the volume of material harvested from the older membranes, so that the total yield was higher.

Comment.—These experiments showed that for mass production of virus, eggs containing embryos preincubated 8 days prior to infection are less satisfactory than those containing older embryos.

B. Pigeon-Pox Virus.

Isolation of pigeon pox virus in hen eggs.

Repeatedly unsuccessful attempts to obtain bacteria-free material from the flesh side of the skin of infected pigeons were made. Beaudette and Hudson (1939) stated that they had had no success with this method but Glover (1939) expressed surprise at their failure.

An attempt was then made to eliminate bacterial contamination from pigeon scab suspensions by passage through the brains of pigeons. (Bierbaum and Gaede 1935, cited by Brandly 1941). The birds usually survived the injection but in no instance was it possible to transfer the virus to eggs.

Penicillin—5,000 units per c.c.—failed to eliminate bacteria from scab suspensions.

Filtration through gradocol membranes was then tried. Ten per cent. suspensions of freshly collected scabs in saline were centrifuged and passed through membranes of 820 $\mu\mu$ average pore diameter. The filtrates were usually sterile but failed to infect either pigeons or eggs.

At this time an article on avian pox in wild sparrows by McGaughey and Burnet (1945) appeared. They found that repeated freezing and thawing of virus suspension prior to filtration gave a filtrate sufficiently active to infect eggs.

A pigeon infected with the D strain of pigeon-pox was killed and the infected follicles removed and ground up with sand. A 20 per cent. suspension of this material was made in broth and then frozen and thawed 5 times in a dry ice-alcohol mixture. It was then centrifuged at 3,000 r.p.m. in an angle-head centrifuge for 20 minutes. The supernatant fluid was passed through a gradocol membrane of 820 $\mu\mu$ A.P.D. This filtrate was injected in 0·2 c.c. amounts on the chorioallantoic membranes of 10 eleven day old embryos, and the eggs were incubated at 35° C. Six days later the eggs were opened. Some showed localised thickenings of the chorioallantois. These were collected for further passage when typical pigeon-pox lesions developed.

The D2 strain was isolated readily in eggs from a filtrate prepared in a similar manner.

Multiplication in eggs.

On the assumption that the conditions found favourable for the propagation of the fowl-pox virus would apply to the pigeon virus, the two strains have regularly been grown on the chorioallantoic membranes of 11 day old embryos at an incubation temperature of 35° C. The membranes were harvested usually on the 4th day after infection. Titration of these membranes in eggs gave end points between 10^{-5} and 10^{-6}. The membranes were thick and white, both at the site of inoculation and at that part of the membrane which forms the base of the true air space. Frequently numerous discrete white areas were seen at the borders of the lesions.

The egg adapted strains of pigeon-pox grew well on scarified breasts of pigeons. Infected follicles became markedly distended but there was little tendency to scab formation. In chickens the strains grew readily and here, too, produced markedly distended follicles. In neither pigeon nor fowls was any tendency observed to spread from the area inoculated.

PREPARATION AND USE OF VACCINES PREPARED FROM EGG-PROPAGATED FOWL- AND PIGEON-POX VIRUS.

A. Fowl-pox vaccine.

After preliminary incubation of 10 to 12 days, eggs were injected on the chorioallantois with 0·15 c.c. of a 1/100 suspension of freshly harvested infected membrane and were re-incubated at 35° C. When stored material was used to infect the eggs early deaths were common; they were, however, less numerous when the seed material was stored in 50 per cent. glycerine.

Six days after injection the entire chorioallantoic membranes were harvested from the live eggs and were disintegrated in Waring blendors. The mush was then suspended in a glycerine-broth vehicle in the following proportions:—

Glycerine—400 parts by volume.

Broth—300 parts by volume.

Membranes—120 parts by volume.

The mixture was bottled in 1 c.c. amounts and stored at 4° C.

Vaccine was issued with instructions that it be used by the single stab method on the lateral aspect of the thigh. A stainless steel stilette 2 inch long and $^{1}/_{16}$ inch in diameter was provided with the vaccine.

Keeping quality and potency of the Vaccine.

Two bottles of fowl-pox vaccine were selected and the activity of the contents measured by titration both in eggs and chickens. One bottle had been stored six months in a refrigerator, the other was only a few days old.

The chickens used in these titrations were about 6 weeks old and were kept under insect-free conditions.

Serial decimal dilutions of the vaccines were made in 50 per cent. glycerine broth for the chicken titrations so that the quantity applied to each bird would be as nearly as possible the same as when undiluted vaccine was used. Two birds were inoculated with each dilution.

Six days after injection the eggs were examined for lesions of the chorioallantois. Smears were made from those membranes which showed doubtful lesions and were examined for Borrel bodies.

The chickens were examined every second day for three weeks. The results of these titrations are shown in table 6.

Result.—Comparative titrations of fowl-pox vaccine in eggs and chickens showed the developing embryo apparently more susceptible to infection than the chicken. From the titrations shown in table 6 and from duplicate titrations it was found that lesions were produced in eggs by suspensions roughly 100 times more dilute than those required to infect chickens.

However, the dose given to each chicken was about 0·005 c.c. while that given to each egg was 0·15 c.c. Thus it requires approximately the same amount of virus to infect the chickens as the egg.

The two batches, 12F and 35F were prepared in the same way and had about the same potency when fresh. It is seen in table 6 that batch 12F had roughly the same value as batch 35F when titrated in eggs and was more active when titrated in chickens. Thus after 6 months storage at 4° C. batch 12F had apparently not dropped in value.

Earlier, a bottle of vaccine, 2F, that had been held 11 months at 4° C. gave an end-point of 10^{-5} when titrated in eggs.

Comment.—These titrations show that the infectivity of fowl-pox virus suspensions for chickens may be evaluated by titration in eggs. As it is frequently difficult to obtain known susceptible birds this is a great convenience.

The titrations also showed that there is apparently no drop in the potency of the vaccine after storage at 4° C. for periods up to 11 months and that stocks of vaccine could be held for this period of time prior to issue.

Reactions produced by fowl-pox vaccine.

Reports from the field and our own observations on birds vaccinated at Onderstepoort showed that almost without exception birds inoculated with fowl-pox vaccine showed takes. Lesions were visible 3 days after vaccination but were fully developed after 12 days when they were about $\frac{1}{4}$ inch in diameter and had yellow crusts. No tendency to spread from the inoculation site was noticed and no undesirable secondary reactions were seen.

TABLE 6.—*Comparative titrations of fowl-pox vaccine in chickens and eggs.*

Batch No.	Dilution.	C.A.M. Lesions.	Chicken Lesions.
12F Stored 6 months at 4 C.....	10^{-1}	+++++	+
	10^{-2}	+++++	+
	10^{-3}	+++	+
	10^{-4}	+++	+
	10^{-5}	+	
	10^{-6}	+	
35F Freshly prepared.....	Undiluted.		+
	10^{-1}		+
	10^{-2}	+++++	+
	10^{-3}	+++++	+
	10^{-4}	++	
	10^{-5}	+	
	10^{-6}	—	

NOTE.—In egg titrations +++++=well developed lesions. In chickens titrations +=a reaction.

Immunity.

At this stage it is not possible to give any detailed information on the immunity produced in the field because of the great difficulty experienced in obtaining reports. No complaints of break-downs were received and small scale exposure experiments at the laboratory indicated that the immunity conferred was solid.

B. Pigeon-Pox Vaccine.

Pigeon-pox vaccine was prepared in very much the same way as the fowl-pox vaccine. Here infected membranes were taken usually on the 4th day after infection and were suspended in glycerine broth in the same proportions as fowl-pox vaccine.

Pigeon-pox vaccine was bottled in 3 c.c. amounts, and was issued with instructions that it be applied by means of a glass rod, $3\frac{1}{2}$ inch long and $\frac{1}{8}$ inch in diameter, to feather follicles exposed by plucking the lateral aspect of the thigh. Three c.c.'s was considered sufficient vaccine for treating 100 birds.

Keeping quality and potency of the vaccine.

Comparative titrations of pigeon-pox vaccine in eggs and chickens were made In these tests one bottle of vaccine that had been held 6 months in a refrigerator, one that had been held four months and one freshly prepared were examined.

The chorioallantoic membranes of the injected eggs were examined for lesions after four days incubation at 35° C. The chickens were examined at two day intervals for three weeks. Where less than 10 follicles were infected they were counted.

The results of thse titrations are shown in Table 7.

TABLE 7.—*Comparative titration of pigeon pox vaccine in eggs and chickens.*

Batch No.	Dilution.	C.A.M. Lesions.	Chicken Lesions.
10P Stored 6 months at 4° C.....	Undiluted.		6+†
	10^{-1}		3+
	10^{-2}	+++*	1+
	10^{-3}	+	—
	10^{-4}	?	
24P Stored 4 months at 4° C.....	Undiluted.		10+
	10^{-1}		8+
	10^{-2}	+++	1+
	10^{-3}	++	
	10^{-4}	+	
	10^{-5}	?	
34P Freshly prepared.....	Undiluted.		10+
	10^{-1}	+++++	10+
	10^{-2}	+++++	1+
	10^{-3}	+++	1+
	10^{-4}	++	
	10^{-5}	+	

* Estimated extent of macroscopic lesion.

† Number of feather follicles infected on each chick : where more than 10 were infected they were not counted, but were given a value of 10.

Results.—From the table it is seen that the developing egg was considerably more susceptible to infection than the chicken. Lesions were produced in eggs by suspensions 100 times less concentrated than those required to infect chickens. The volume of vaccine dilution applied to each chick was estimated at 0·03 c.c. while that deposited on the membrane of each egg was 0·15 c.c. It thus appeared that approximately 20 minimal egg-infecting doses are required to infect the chick.

The three batches were prepared in the same way and probably had the same potency when fresh. That is, all contained 1,000 minimal chick infecting doses per 0·03 c.c. of vaccine (batch 34P). It appears from table 7 that the virus titre of batch 24P after 4 months storage at 4° C. had fallen to 100 M.I.D.'s and after 6 months storage that of batch 10P had fallen to about 10 M.I.D.'s The egg titrations showed a corresponding difference in the potency of the three batches:—

> 100,000 M.I.D.'s in the freshly prepared batch 34P; 10,000 in the four months old batch 24P and 1,000 in the six months old batch 10P.

Comment.—The titrations showed that here too, there is a correlation between titrations made in eggs and chickens, and that the egg may, therefore be used to estimate the virus titre of this vaccine.

The titrations also show that there is a considerable drop in titre of the vaccine on prolonged storage. Provided, however, the vaccine contains 1,000 minimal chick infecting doses when fresh, storage up to 6 months is feasible.

In a personal communication, de Blieck (1947) stated that, in his experience, pigeon-pox vaccine prepared from egg cultivated virus and made up in fluid form is rapidly inactivated at room temperature. To check this observation a number of bottles of pigeon-pox vaccine that had been held a month at 4° C. were placed in an incubator running at 32° C. Four days later a bottle was tested on 25 eight-week old chicks. All showed good reactions. Another bottle was tested after 16 days in the incubator. About half the birds reacted and the takes were poor.

Reactions produced by the vaccine.

All birds vaccined with pigeon-pox vaccine that have been examined showed takes. Infected follicles became greatly distended. At first they were red in colour but later become yellowish and frequently yellow plugs were found in the opening of the follicles. Maximum development occurred about 10 days after vaccination.

No tendency to spread and no systemic reactions have been noticed.

Immunity.

Work on the immunity conferred by pigeon-pox vaccine is being continued. From the results obtained and from field reports it appears that the degree of immunity conferred is variable. The indications are that roughly half the birds vaccinated become fully immune to fowl-pox virus but in others immunity varies from almost complete to practicaly none at all, even where the reaction to the pigeon virus had been marked.

SUMMARY.

1. Fowl-pox as it occurs in the Union of South Africa is reviewed briefly together with relevant literature on the methods of immunization.

2. In South Africa until recently vaccine prepared from virus propagated on pigeons has been used for immunization of poultry. The reasons for abandoning this vaccine are discussed.

3. The technique of propagating fowl- and pigeon-pox virus in eggs is discussed, attention being paid to:—

(*a*) The influence of temperature of incubation;
(*b*) The time allowed for multiplication;
(*c*) The distribution of virus in the eggs;
(*d*) The virus titre of infected membranes;
(*e*) The isolation of pigeon-pox virus strains.

4. The preparation of fowl- and pigeon-pox virus vaccine is described with particular reference to:

(*a*) Potency of the vaccine and its estimation;
(*b*) The keeping quality;
(*c*) The correlation between infectivity for egg and for chickens.

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