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Bacillary White Diarrhoea of Poultry and its Eradication in the Union of South Africa.

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BACILLARY WHITE DIARRHOEA and fowl typhoid, are the two diseases that cause the greatest mortality among the flocks of poultry in the Union.

Martinaglia (1927) recorded for the first time a definite diagnosis of bacillary white diarrhoea in the Union in chickens, and by means of the agglutination test picked out four reacting fowls from a group of six birds that had laid the eggs from which the chicks had been hatched.

In December, 1936, at the annual meeting of the South African Veterinary Medical Association, Coles introduced his suggested plan for undertaking large-scale testing in the Union. Although a number of changes in this original plan have taken place, as the result of experience gained, the main structure remains.

The plan of eradication now being carried out is embodied in the agreement form, which poultrymen are compelled to sign if they wish to have their birds tested. It contains thirteen clauses, and a copy of the form is appended. (See appendix.) Comment on some of the clauses is necessary:—

Clause 1.—For the convenience of poultrymen special rings are procurable from the South African Poultry Association. These are to enable the veterinary officer to identify birds after test. Once the ring is put on the leg another bird cannot be substituted for it.

Clause 2.—Blood may be taken " by a person authorised by the Director of Veterinary Services ". Anyone who wishes may qualify to be authorised by sending 100 samples of blood from fowls he has himself bled. If these samples are suitable for testing purposes he is issued with a certificate by the Director of Veterinary Services. This entitles him to bleed fowls or turkeys for anyone who wishes to have his flock of birds tested. He is also entitled to charge 1d. a bird for his work. Students of the Faculty of Veterinary Science are often used for this purpose. It should also be noted that all birds of four months of age and older must be submitted to testing. Under exceptional circumstances, and only for diagnostic purposes, may a portion of a flock be tested. No individual results are given the poultryman in these cases. He is informed that the disease is either present or not. Birds intended for export are always tested by the Department. This is in order to comply with regulations of the adjoining territories, to which the birds may be exported. A copy of the certificates is appended.

Clause 3.—Originally a flock of birds had to give two consecutive negative tests at an interval of a month. As a result of experience it was found that if all birds of a flock gave complete negative test positives were rarely, if ever, found on the second test. A Bacillary White Diarrhoea Free Certificate is now given to a poultryman if his entire flock is clean at the first round of testing. If positive reactors are found, all birds must still give two clean consecutive negative tests before a Free Certificate is issued.

Clauses 5 *and* 6.—These conditions were drawn up to prevent infection from being introduced on to a clean poultry plant.

Clause 8.—This refers to the compulsory destruction of all positive reactors. The positives, after being checked, must be destroyed, and the carcasses are handed back to the poultryman for disposal as dressed poultry. As a concession to poultrymen, if they are long distances from the nearest veterinary officer, and to assist this official, the leg of the bird with the legband intact is accepted as proof that the bird has been destroyed. Under no circumstances are positive reactors allowed to be sold as live poultry, except where they are brought to the shop of a dressed poultry merchant, and are there checked by a veterinary officer or his representative prior to slaughter.

Clause 10.—This is very necessary in order to protect purchasers. It was found that some people, whose birds were still under test, insert phrases such as " B.W.D. tested ", " All birds blood tested ", " B.W.D. tested stock ", " Stock from B.W.D. tested birds ", in their advertisements, and many would-be purchasers were thereby misled into thinking that the birds were bacillary white diarrhoea free.

Clause 11.—This was inserted because on different occasions *S. anatum*, *S. typhi-murium*, and *S. enteritidis* (var. Dublin) have been isolated from fowls in the Union. Our experiences were borne out by McGaughey (1932), Jansen (1936), Henning (1937 and 1939).

Collection of Blood Samples for Testing Purposes.

Blood is drawn from the vena brachialis, which is easily seen under the skin on the ventral surface of the extended wing. A medium-sized hypodermic needle is used in preference to a surgical lancet, although this last instrument is better for bleeding pigeons, (Haig, 1940). The blood is collected in glass tubes 8 cm. long with a diameter of 8 mm., and corked at both ends. Each tube contains 0·5 c.c. of a 2·5 per cent. boracic acid solution, which acts as a preservative. These tubes fit into pockets of a special calico " apron " and each apron holds 10 tubes. The pockets of the aprons are marked, starting from 1 to 10, 11 to 20, and so on. Ten aprons comprising one unit are fitted into a fairly thick cardboard box 17·5 cm. by 14·5 cm. by 10 cm. On the lid and underneath this box a stencilled number is placed.

Accompanying each box of 100 tubes are four forms; one form is in triplicate, and a fourth is single. These are printed in both official languages of the Union. The form in triplicate gives the number corresponding to the box, the name of the owner of the birds, his address, the bleeder's name,

when the birds were bled, when the blood was despatched, and how many samples were sent. On arrival at the laboratory it is stamped on the day of receipt. Below this information is a rectangular ruled area, divided into 100 squares. Each number in the spaces corresponds to a tube in a similar number on the apron so that space 1 represents tube 1, space 22 represents tube 22, and so on. The fourth form contains a large rectangle 16 cm. by 13 cm. also divided into 100 large spaces. These spaces are also numbered 1-100, and again each number corresponds with the similar number in the apron. These spaces are for the insertion of the leg or wingband numbers. These forms are reproduced in the appendix.

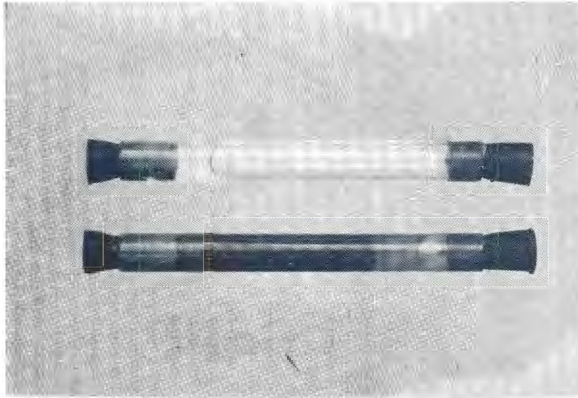


Fig. 1.—Two tubes with corks showing, one tube with preservative and one with coagulated blood and serum.

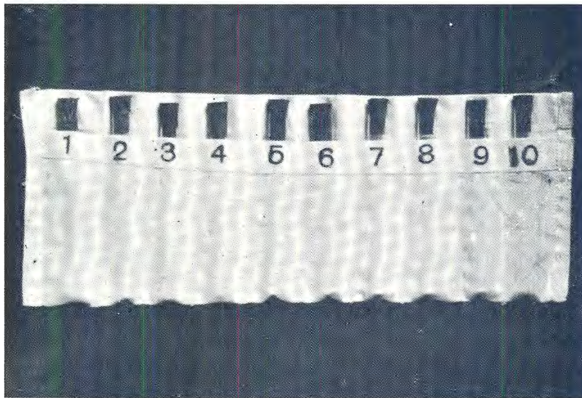


Fig. 2.—Apron containing ten tubes and showing numbers.

In order to obtain good serum it is advisable to keep the tubes of blood at a temperature of at least 80° F. If the temperature, where the bleeder is working, is over that mark, no artificial heat is necessary. If not, the tubes, in batches of ten, as the birds are bled, are placed in an ordinary small

incubator, running at about 97° F, sometimes placed near the stove in the kitchen, or in an incubator box, designed by Coles, and which is most effective. A photograph of this box with descriptions appears in the appendix.

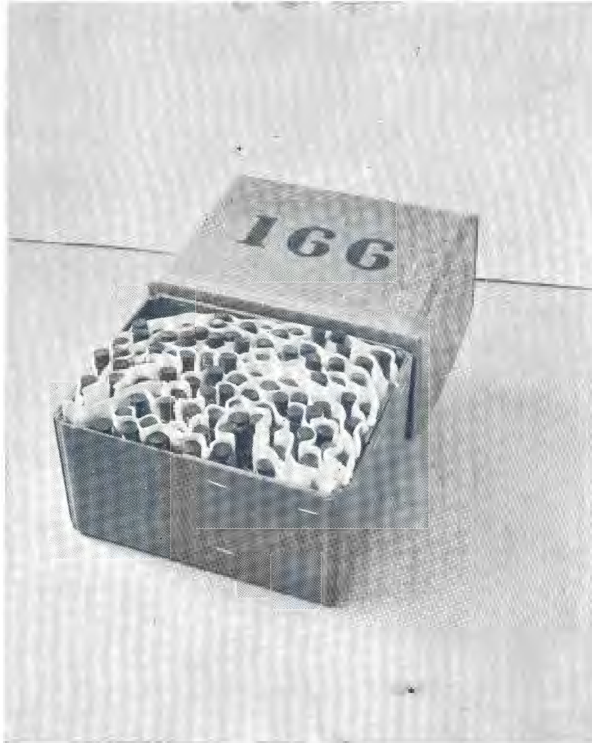


Fig. 3.—One unit showing numbered cardboard container made up of ten aprons each holding ten tubes.

An hour later the cork, to which the clot of blood is attached in each tube, is twisted. This detaches the clot from the side of the tube, and good clear serum is expressed. There are a number of advantages in using the sort of equipment described. Considering the tubes first, the double corks help in loosening the blood clot to obtain good serum. The serum can always be reached, no matter at what end of the tube it has been expressed. The number of useless samples is negligible, and the tubes can be easily cleaned. Rarely, if ever, are tubes broken in transit. The linen aprons keep the tubes together, yet not in direct contact, thus saving breakages. They are small, cheap, and compact, and can be easily washed each time they are returned. The rubber corks are ideal for boiling and cleaning, as they are smooth. Ordinary corks do not stand boiling and, as they frequently have cracks in them, it is impossible to get masses of coagulated blood from the cracks. The cardboard boxes are small, and even with 100 tubes in them, are light in weight, thus saving excessive amounts of postage on them, when despatched from the farm to the Laboratory. Finally, they are very durable; many boxes at present in use have been utilised for over five years.

As a preservative 0.5 c.c. of a 2.5 per cent. boracic acid solution is used for each tube, and in every box two small bottles of about 50 c.c. of the same preservative is added in case leakage from any tubes takes place. The serum remains good for at last six days if the weather is cool, and for four days on the average in really warm weather. This means that no place in the Union is too far away for the testing centre at Pietermaritzburg, Natal.

The Test.

Square metal stands, holding ten narrow racks each, with holes for ten tubes, are used. Each rack is painted black with small white numbers which range, according to the rack, from 1 to 100. Thus one stand of one hundred tubes takes all the samples of one box. In order to accomodate varying sized tubes, and to prevent their falling out of the racks, a narrow wooden ledge, with depressions for the ends of the tubes, is attached.

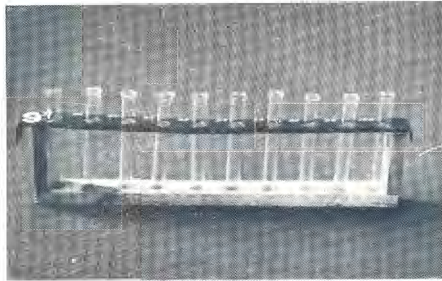


Fig. 4.—Single rack with tubes.

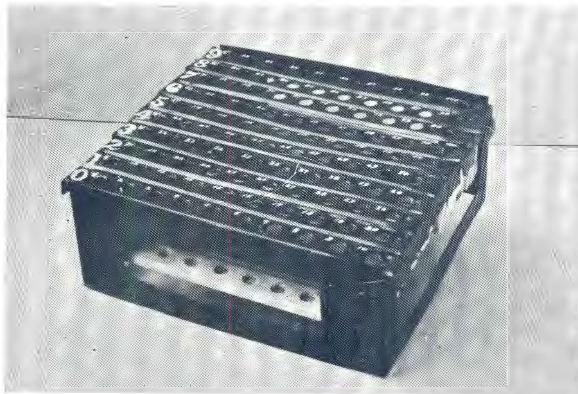


Fig. 5.—Square metal stand holding ten racks suitable for testing one cardboard container full of one hundred blood samples. Numbers on racks correspond to numbers on aprons.

To obtain the right amount of serum for tests, glass tubing of a very small calibre is used. According to its calibre, definite lengths are cut for each size. Each individual length holds 0·1 c.c. of serum which is added to 1 c.c. of antigen. Needless to say, only one length of tubing is used for one

sample. At the end of the day these capillary tubes are all washed through, boiled, and dried in a hot-air oven ready for use the next day. A small dome-shaped rubber teat is attached to each length of tubing, for sucking up the serum and ejecting it into the tube of antigen. As each rack of ten tubes is filled, each tube is shaken to obtain a proper mixture of serum and antigen. The tests are placed in an incubator for about eighteen hours and then read.

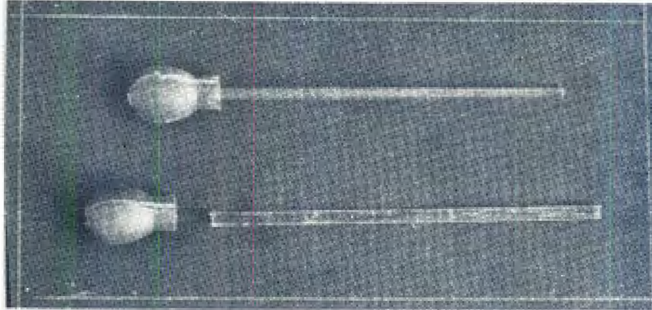


Fig. 6.—Capillary tubing with small dome-shaped rubber teats for sucking up serum.

The symbols entered on the accompanying forms over the small numbered squares, are X for positive, O for useless and N for no specimen. The form in triplicate is then filled up. One form goes to the owner, another goes to the local Government Veterinary Officer, and the third form is retained for the poultryman's file at the Laboratory. The legband form is also retained here.

Finally, where positives occur, a special report bearing the actual legband numbers of the reactors is sent to the Senior Veterinary Officer of the Province. This is retransmitted by him to the local Veterinary Officer, who uses this information when checking positive birds prior to their destruction.

Clean Flocks.

When a flock of fowls has been declared free of bacillary white diarrhoea and fowl typhoid by testing, the poultryman is issued with a Government Free Certificate, which is valid for twelve months only. To retain the certificate, all birds must be tested annually. In the case of a certificate holder doing his annual test, if all his birds are clean, a second round of testing is not insisted on and he is issued with a new certificate for the next twelve months. Should a holder of a free certificate find any reactors at an annual test— and this often occurs at the annual test twelve months after receipt of the free certificate—he has to obtain clean tests before receiving another free certificate. The finding of reactors,, twelve months after a completely negative test, has been the experience of workers in different parts of the world and the reason has been clearly explained by Beaudette and Black (1926).

Appearance of Infection in Flocks Previously Negative.

Breakdowns in clean flocks are to be deplored but occur wherever large-scale testing is practised. One understands the carry over of infection through pullets the year after a flock has been declared free, but where infection takes place after a flock has had two or three consecutive clean tests it is much harder to explain. A searching enquiry frequently elicits no useful information.

Conditions under which Testing takes place.

Up to 1943 anyone in the Union could have his fowls tested by the Department, provided he signed the agreement form. This resulted in a spate of applications. Many of the big poultry breeders had flocks up to 2,000-3,000 birds, while a very large number of other applicants had between 100 and 200 birds. Clearing up of the big poultrymen's flocks was not difficult, but, in spite of repeated tests extending over a number of years, many of the small flocks showed as many reactors at the end as at the beginning of the test. On investigation it was found that many of these small poultry keepers were what might be termed "backyard farmers". The poultry houses were deplorable, many vermin-infested, the floors of soil, and the runs had had fowls in them for years. The houses were rarely cleaned and no hygiene at all was practised, while on some the birds simply ran all over the farm and roosted in the trees at night.

The present policy is quite different. Anyone wishing to have his fowls tested makes application to the Laboratory. The application is then forwarded to the Senior Veterinary Officer of the Province. His representative then visits the plant of the applicant and examines the whole lay-out. If it is up-to-date, has good houses with impervious floors, good hygiene is practised, and a good class of bird is kept, he reports favourably on it, giving in addition the class of farming practised, intensive or semi-intensive, and the total number of birds on the plant. The applicant is accepted, and only when proof is produced will the South African Poultry Association sell him bacillary white diarrhoea rings.

If a person is not accepted he is informed of the reason why and suggestions are made to help him improve his plant. As a result of adopting this policy, it is hoped that many more of the up-to-date poultrymen will be induced to adopt the bacillary white diarrhoea testing scheme. It must be clearly understood that the bacillary white diarrhoea scheme in the Union is at present entirely voluntary; there is no compulsion on the part of the Department for people to join it.

GENERAL.

Testing, by means of the tube method, commenced in the Union when two poultry farmers in the Ermelo district sent in the first sets of samples to Onderstepoort in 1937. About March 1938 testing was opened to the public.

From then on to the present time more and more farmers have asked for their birds to be tested. Each year from 1938 on, more farmers joined the testing scheme, but in 1944 saw a rise in tests from 129,674 to 216,616. A graph is given showing the annual numbers of tests done. (See appendix.)

The present number of holders of the Government Bacillary White Diarrhoea Free Certificate is 151 as against 72 in 1943. Divided into the different Provinces, the position is as follows:—

Natal	44 holders.
Cape	22 holders.
Transvaal	64 holders.
Orange Free State	21 holders.

In addition a further sixty farms are still being tested for their free certificate for the first time.

Discussion.

Looking at the progress of the testing of poultry for bacillary white diarrhoea in the Union of South Africa, it was at first slow but was maintained and there was a gradual increase in the number of tests done annually for the first five years followed by a marked increase in the number of tests done in 1944.

The bacillary white diarrhoea scheme adopted in the Union is the strictest in the world, but the results already achieved, fully justify the terms of agreement that were drawn up and are now in force. Above all, the scheme is a voluntary one.

Experimental Work.

Antigen.

(1) *Strains of Organisms.*

Strains of both *S. pullorum* and *S. gallinarum* were obtained from birds sent in for post-mortem examination or from reactors picked out by testing. These birds emanated from widely separated parts of South Africa. Cultures were made from heart's blood, spleen, bile and ovaries. Single smooth colonies were picked and the organisms were identified in the usual manner. In the case of the isolation of organisms from the ova it was found advisable to pass them through tethrathionate broth prior to plating on MacConkey's medium. Cultures of these two salmonellas were often obtained from the bile when cultures made from heart's blood and spleen were negative.

In order to obtain pure strains for stock purposes the method of selecting smooth colonies, as described by Henning (1939), was adopted. In no case was any strain obtained by single-cell isolation.

(2) *Standardization.*

From the beginning of this work in South Africa it was decided to test out all strains of *S.pullorum* and *S. gallinarum* and antigens, to be used for testing purpose, against a serum specially prepared from sheep that were immunised against *S. gallinarum*. The titre aimed at was between 1-800 and 1-1,000 and this was called the Allerton standard. By this means one would be able to compare results, as all antigens were standardized.

The preparation of the standard serum followed the stereotyped methods closely. Killed cultures of a strain of *S. gallinarum* made up to different densities were injected intravenously at definite intervals. From 23 to 25

days from the time of the first injection the sheep were bled, the blood was allowed to coagulate and under aseptic conditions the expressed serum was decanted into small bottles. These were sealed and stored in the refrigerator. The first batch of serum was kept in small bottles in a dark refrigerator and the titre was maintained for about six months.

It was suggested that if the serum was dried its high titre would probably be retained, or at least it would retain a high titre for a longer period than in the fluid state.

Fresh serum was sent to Onderstepoort, and the work was kindly done by Dr. Alexander. The method used was as follows: The serum was accurately measured off into 1·2 c.c. amounts, degassed at a negative pressure of 1 cm. of mercury for 4 hours, rapidly frozen by immersion in an alcohol—carbon dioxide snow mixture at a temperature of approximately -70° C. and then dried over anhydrous calcium sulphate at a negative pressure of 0.001 mm. of mercury for 48 hours. The ampoules containing the dried serum were sealed in an atmosphere of dried air, not *in vacuo*. The serum was reconstituted here by the addition of 1·2 c.c. of recently-boiled cooled water. This dried serum retained its high titre for about one year.

Most single strains isolated, when titred against positive sheep serum, give reactions in dilutions of from 1:1000 to 1:1600. A small number, on the other hand, give reactions from 1:100 to 1:400. Unless a strain gives a positive titre of from 1:1000 to 1:1600 it is discarded.

At the beginning of this work the negative serum used in the control tests was obtained from sheep and for a time appeared quite suitable. As a result of certain reactions that occurred during trial testing it was found that some strains, which might be termed ultra-sensitive, gave a negative reaction with negative sheep serum. In a dilution of 1:10, when tested against known negative fowl serum, they give an almost complete positive reaction. As a result of this finding known negative fowl serum is always used for control purposes.

A possible explanation of this phenomenon is to be found in the fact that some strains, biologically classical strains of *S. pullorum* and *S. gallinarum*, when tested in dilution of from 1:2 to 1:10, using known negative fowl serum, will frequently give positive reactions in dilutions of 1:3 to 1:4, some up to 1:6, and in the writer's collection is a strain P316 that gives a positive reaction in a 1:12 dilution. A few examples are given. (Table 1.)

Rebrassier (1926) stated that the normal agglutination titre of normal fowl blood was 1:10. From this investigation and the testing of hundreds of thousands of fowls no definite evidence has been found to confirm his statement. This is in accord with the findings of Doyle (1925).

Summary:—

(1) Only smooth (S) single colonies should be picked as a preliminary step in the isolation of any strain.

(2) Standardization of all strains should be carried out prior to their utilization for testing purposes.

(3) A method of preparing a standard serum, and also of preserving it in a dried state is described.

(4) Most strains of *S. pullorum* and *S. gallinarum* when tested with the Allerton standard strain serum were agglutinated by dilutions of 1/1000 to 1/1600 of the serum.

(5) Known negative fowl serum should be used as a negative control.

(6) Low dilution titrations from 1:3 to 1:10 with known negative fowl serum should be carried out with all strains of either *S. pullorum* or *S. gallinarum* prior to using such strains for the preparation of antigens intended for routine testing purposes.

TABLE 1.

Strains.	1 : 3	1 : 4	1 : 5	1 : 6	1 : 7	1 : 8	1 : 9	1 : 10	
G.4554......	+	–	–	–	–	–	–	–	
G.4840......	++	+	–	–	–	–	–	–	
P.594........	+	–	–	–	–	–	–	–	
P.94.........	++	++	–	–	–	–	–	–	
P.216........	–	–	–	–	–	–	–	–	
P.4779.......	++	–	–	–	–	–	–	–	
P.5206.......	++	–	–	–	–	–	–	–	
G.510.......	–	–	–	–	–	–	–	–	
P.5133.......	++	++	++	++	–	–	–	–	
P.5479.......	++	++	–	–	–	–	–	–	
G.4371......	–	–	–	–	–	–	–	–	
G.129.......	–	–	–	–	–	–	–	–	
P.316........	++	++	++	++	++	++	++	++	1 : 12 ++

(3) *Preparation of antigen.*

At the commencement an ordinary nutrient agar medium, which was adjusted to a pH of 7·4 was used. On this medium *S. pullorum* gave only a fair growth, while *S. gallinarum* gave heavier growths. Because the growths were not heavy as was hoped for, it was discarded in favour of a liver medium. This was the medium used in the work of Stafseth and Thorp, Jnr. (1928). The composition of the liver medium was as follows:—

Water: 4000 c.c.

Minced liver: 3 Kg.

This was boiled for about 45 minutes, and to it was added—

Sodium phosphate: 8 gm.

Sodium chloride: 20 gm.

This was now strained through a flannel and adjusted to a pH of 7·2. Finally was added—

Agar: 130 gm.

Glucose: 40 gm.

This was poured into Roux flasks, sterilized, and was then ready for seeding.

At the beginning peptone was added as well but it was found that the growth of *S. pullorum* or *S. gallinarum* was so adherent that much of it could not be removed by washing. An attempt to obtain a luxuriant growth was made by adding varying quantities of ox bile, but it was found that this ingredient had no effect in increasing the growth. After further experimentation it was finally decided to add 8 gm. or 0·2 per cent of sodium citrate to the liver medium previously described. This was in view of the findings of Bushnell and Hudson (1927). The growth of *S. pullorum* on this medium is very fair, some strains yield a bigger harvest than others while *S. gallinarum* gives fairly heavy growths.

Prior to washing off growths from the Roux flasks, all water of condensation is poured off. The reason for this will be given further on.

For washing off the organisms from the medium, sterile physiological saline solution, to which 0·2 per cent phenol has been added, is used. The washings are filtered through gauze into large flasks and the filtrate is then centrifuged for half an hour at 3,000 r.p.m. The clear supernatant liquid is discarded, and enough fresh carbolized saline is added to make a thick emulsion of the organisms. The thick emulsion is now stored for later use.

Having established a basic method of preparation, experiments were next carried out to ascertain whether this method could not be simplified further.

Antigens were made up of strains of *S. pullorum* and *S. gallinarum* respectively. Five different methods of antigen preparation were tried:—

A. The organisms were washed off the medium with carbol-saline solution, the water of condensation, being retained. The antigen was unfiltered and not sedimented by centrifuging.

B. The water of condensation was poured off and the organisms washed off with carbol-saline solution. After filtration and sedimentation a concentrated suspension with fresh carbol-saline solution was made.

C. The water of condensation was retained, the organisms were washed off with carbol-saline solution, filtered through muslin, sedimented and made into a concentrated suspension with fresh carbol-saline solution.

D. The water of condensation was discarded, the organisms were washed off with carbol-saline solution, not filtered, nor sedimented by centrifugation.

E. The water of condensation was retained, the cultures washed off with carbol-saline solution, not filtered, sedimented and made into a concentrated suspension with fresh carbol-saline solution.

These different antigens were tested with standard serum roughly every month for eleven months in the case of *S. pullorum* antigens, and for ten

months in the case of *S. gallinarum* antigens. The serum used was the standardized sheep serum previously referred to. The results are here tabulated (Table 2). See graphs 1 and 2.

TABLE 2.

S. pullorum.

Method.	Water of condensation.	Filtration.	Sedimented by centrifuge.	Resuspended in carbol-saline.	Results of storing.
A..........	Retained.	No.	Not.	Not.	Value dropped rapidly.
B..........	Discarded.	Yes.	Yes.	Yes.	Value retained well.
C..........	Retained.	Yes.	Yes.	Yes.	Value retained fairly well.
D..........	Discarded.	No.	Not.	Not.	Value retained fairly well for six months followed by rapid fall.
E..........	Retained.	No.	Yes.	Yes.	Value retained fairly well.

S. gallinarum.

Method.	Water of condensation.	Filtration.	Sedimented by centrifuge.	Resuspended in carbol-saline.	Results of storing.
A..........	Retained.	No.	Not.	Not.	Value dropped gradually.
B..........	Discarded.	Yes.	Yes.	Yes.	Retained fairly constant value.
C..........	Retained.	Yes.	Yes.	Yes.	Maintained a low value.
D..........	Discarded.	No.	Not.	Not.	Value dropped fairly quickly.
E..........	Retained.	No.	Yes.	Yes.	Maintained a low value.

Discussion.

S. pullorum.—From the graph (see appendix) it will be seen that B. antigen maintained its value the best— this is the method at present used for preparing antigens for routine purposes. A. antigen, which was simply a washing off of the organism from the media, gave the worst results as the value declined very rapidly. C. and E. antigens were prepared almost similarly to B. antigen. The water of condensation was poured off in B. antigen prior to washing off the organisms with carbol-saline solution, while in antigens C. and E. this water was poured off together with the carbol-saline solution used for washing off the growths after sedimentation by the centrifuge. D. antigen could for all practical purposes be classed with A. antigen.

S. gallinarum.—With the various antigens it was noted that at the time of preparation all showed a lower value than did the pullorum antigens. The decline in value was more rapid than in the case of the pullorum antigens. The fall in value shown by the *S. gallinarum* antigens resembles closely the fall obtained with the *S. pullorum* antigens. Antigens A. and D. could be grouped and antigens C. and E. Antigen B. maintained its titre the best.

Summary.—It would appear that the retention of the water of condensation tends to lower the sensitivity of antigens, which makes them unreliable for testing purposes within six to eight months from the time of preparation. The water of condensation should, therefore be poured or siphoned out of a flask before washing off the growths for the preparation of test antigens.

(4) *Keeping qualities of antigens.*

From various batches of *S. pullorum* and *S. gallinarum* antigens, prepared from three or four strains of the different organisms for routine testing, samples were kept and tested on different occasions for over a period of two years.

S. pullorum antigens.—At the time of preparation their agglutination with standard serum varied from 1:600 to 1:1000. In most cases their values were maintained with a gradual drop for almost two years. Some dropped until a dilution of 1:400 was reached, when they remained at this figure up to the close of the experiment. At the end of the experiment all these antigens were tested against the same known positive fowl serum, and gave positive results in the same dilution of serum as did freshly-prepared antigens against the same positive fowl serum. All titrations were carried out by the tube test and the dilutions varied from 1-10 to 1-320. (See graph 3).

S. gallinarum antigens.—In this case results were different from *S. pullorum*. The commencing reactions were somewhat higher varying from 1:800 to 1:1200, but within three months had dropped considerably, some even going as low as 1:200. After this drop they appeared to increase in value and then became more or less stationary, but still at a low figure when compared with their original value. Antigens showing a reaction with serum of 1:400 or more when tested against known positive fowl serum in dilutions of from 1:10 to 1:320, gave good positive reactions.

Antigens below 1:400, and more than 1:200, when tested against the same positive fowl serum, gave only suspicious or negative reactions in many cases.

Finally, antigens with a reaction of 1:200 and less gave consistently negative results with known positive fowl serum. This deterioration took place quicker with some antigens, but in a little over a year many had deteriorated. (See graph 4).

Discussion.—(*a*) *S. pullorum* antigen appears to be a more stable product than does *S. gallinarum* antigen.

(*b*) *S. pullorum* antigens with a commencing reaction with serum of from 1:800 to 1:1000, if kept at icebox temperature, will maintain a value of at least 1:400 for at least two years, and still give reliable results for testing purposes.

TABLE 3.

Antigens.—Dilutions with Allerton Standard Sheep Serum.

Strain.	1/100	1/200	1/400	1/500	1/600	1/720	1/800	1/1000	1/1200	1/1600	
S1	++	++	++	++	++	++	++	++	+	—	Negative Controls-neg.
S2	++	++	++	++	++	++	++	++	++	++	,, ,, ,,
S3	++	++	++	++	++	++	+	—	—	—	,, ,, ,,
S4	++	++	++	++	++	++	+	—	—	—	,, ,, ,,
S5	++	++	++	—	—	—	—	—	—	—	,, ,, ,,
S1+S2	++	++	++	++	++	++	++	++	++	—	,, ,, ,,
S1+S3	++	++	++	++	++	++	++	—	—	—	,, ,, ,,
S1+S4	++	++	++	++	++	++	+	—	—	—	,, ,, ,,
S1+S5	++	++	++	++	++	++	++	—	—	—	,, ,, ,,
S2+S3	++	++	++	++	++	++	++	+	—	—	,, ,, ,,
S2+S4	++	++	++	++	++	++	+	—	—	—	,, ,, ,,
S2+S5	++	++	++	++	++	+	—	—	—	—	,, ,, ,,
S3+S4	++	++	++	++	++	++	—	—	—	—	,, ,, ,,
S3+S5	++	++	++	++	+	—	—	—	—	—	,, ,, ,,
S4+S5	++	++	++	++	—	—	—	—	—	—	,, ,, ,,
S1+S2+S3	++	++	++	++	++	++	++	+	—	—	,, ,, ,,
S1+S2+S4	++	++	++	++	++	+	—	—	—	—	,, ,, ,,
S1+S2+S5	++	++	++	++	++	+	—	—	—	—	,, ,, ,,
S2+S3+S4	++	++	++	++	++	—	—	—	—	—	,, ,, ,,
S2+S3+S5	++	++	++	++	++	+	—	—	—	—	,, ,, ,,
S3+S4+S5	++	++	++	++	++	—	—	—	—	—	,, ,, ,,
S1+S3+S4	++	++	++	++	++	—	—	—	—	—	,, ,, ,,
S1+S3+S5	++	++	++	++	++	+	—	—	—	—	,, ,, ,,
S1+S4+S5	++	++	++	++	++	—	—	—	—	—	,, ,, ,,
S2+S4+S5	++	++	++	++	++	++	—	—	—	—	,, ,, ,,
S1+S2+S3+S4	++	++	++	++	++	—	—	—	—	—	,, ,, ,,
S1+S2+S3+S5	++	++	++	++	++	+	—	—	—	—	,, ,, ,,
S1+S3+S4+S5	++	++	++	++	++	++	—	—	—	—	,, ,, ,,
S2+S3+S4+S5	++	++	++	++	++	—	—	—	—	—	,, ,, ,,
S1+S2+S3+S4+S5	++	++	++	++	++	—	—	—	—	—	,, ,, ,,

All these S. strains were *S. pullorum* strains.

(*c*) *S. gallinarum* antigens if kept for more than a year at icebox temperature, should be standardized frequently and tested against known positive fowl serum.

(*d*) Any antigen, whether *S. pullorum* or *S. gallinarum*, should be discarded for testing purposes if the reaction drops below 1:400 (Allerton standard serum.

(5) *Single Strain Antigens as Compared with Antigens Composed of Several Strains.*

As mentioned previously, single strains of *S. pullorum* or *S. gallinarum* vary in reaction from 1:100 to 1:1600 or more by the Allerton standard. When a number of strains of either *S. pullorum* or of *S. gallinarum* are mixed to form either a *pullorum* or *gallinarum* antigen for test purposes, it will be found that the values of such antigens will be reduced to a figure between that of the highest and lowest strain and, as time elapses, falls still lower as has already been shown. Table 3 gives examples.

These highly agglutinable single strains were used to subcheck test results carried out with antigens composed of more than one strain of either *S. pullorum* or *S. gallinarum*. In many cases more reactors were picked out with the single strain antigens. The test dilutions were similar in all tests. These results were further sub-checked by submitting the same positive serum to a number of high valued single strain antigens

The result might be ascribed to the greater sensitivity of the single strains.

To prove that the birds, which gave a positive reaction to single strain antigens and a negative reaction to antigens made up of more than one strain, were actually positive, many were selected and a bacteriological examination was carried out. In the majority of cases either *S. pullorum* or *S. gallinarum* was obtained from them.

Boxes each containing 100 samples of sera, from known infected farms, were taken and parallel tests were carried out on them using two different single strain antigens and an antigen made up of more than one strain. Table 4 shows the total positives in each box.

Both S. and P. were *pullorum* antigens.

These examples are taken from a large number of comparative tests carried out and are typical as regards the discrepancy between single and multiple strain antigens. This is especially the case when testing a flock that is badly affected. Where there are only a few reactors the discrepancy is not so marked. The discrepancies even between each single strain should be noted. A number of birds missed by the multiple strain antigens were left in the flocks and within a month, i.e. the next round of testing, they were picked out by a multiple strain antigen or, at the latest, after two months.

In most cases, not all, these figures further confirmed the view, that the highly agglutinable antigens pick out more positive carriers than do antigens of lower value. The exceptional cases, e.g. 478. b. and 267. a. bear out the findings of May and Goodner (1926) who stated " the fact that some antigens will agglutinate in a given serum as much as eight times

as well as others explains some of the results, namely, that some antigens will usually detect more reactors than others; but the fact that the weaker agglutinating antigens will in certain flocks detect more reactors than the more readily agglutinated strains still remains unexplained ".

TABLE 4.

No. of box.	Single strain antigen and its value.	No. of positives.	Multiple strain antigen and its value.	No. of positives.
286. a.............	S.1. (1 : 1,200).	47	P.77. (1 : 800).	41
286. b.............	S.2. (1 : 1,600).	49	P.77. (1 : 800).	41
527. a..............	S.1. (1 : 1,200).	13	P.78. (1 : 800).	8
527. b.............	S.2. (1 : 1,600).	18	P.78. (1 : 800).	8
195. a.............	S.2. (1 : 1,600).	48	P.78 (1 : 800)..	25
195. b.............	S.4. (1 : 1,000).	38	P.78. (1 : 800).	25
478. a.............	S.1. (1 : 1,200).	73	P.78. (1 : 800).	48
478. b.	S.5. (1 : 500).	66	P.78. (1 : 800).	48
267. a.............	S.4. (1 : 1,000).	71	P.77. (1 : 800).	81
267. b.............	S.3. (1 : 600).	78	P.77. (1 : 800).	81
380. a.............	S.2. (1 : 1,600).	78	P.77. (1 : 800).	69
380. b.............	S.1. (1 : 1,200).	75	P.77. (1 : 800).	69
107. a.............	S.1. (1 : 1,200).	11	P.83. (1 : 720).	8
107. b.............	S.8. (1 : 1,200).	10	P.83. (1 : 720).	8
256. a.............	S.7. (1 : 1,600).	19	P.83. (1 : 720).	19

Discussion.—(*a*) The best antigens are obtained by using single strains for antigen production.

(*b*) The values of antigens are reduced when more than one strain of the organisms are utilized.

(*c*) In most cases more positive reactors are picked out in badly infected flocks when single strain antigens are used in preference to antigens made up of more than one strain.

(*d*) It appears that in most cases the higher the value of the antigens, the more positive reactors are picked out, but with Allerton standard the agglutination should not exceed 1:1600.

(*e*) In a small number of cases a lower valued antigen may pick out more reactors than an antigen with a high value.

(*f*) From the practical point of view, in order to reduce the number of rounds of testing to clear a flock of *S. pullorum* or *S. gallinarum* carriers a single strain antigen of high value should be used for testing purposes.

(6) *Flocculation and Cloudy Reactions in Antigens.*

These peculiar reactions appeared in tests here from the beginning, but have now for all practical purposes been overcome.

Observations made here show that while a few sera give either the cloudy reaction or flocculation with antigens during testing throughout the year, the majority of affected sera are found from about December to May of each year. This period is the rainy season and the growth of grass is good. This is also a period when egg-laying is high. These affected sera have either a marked bronze or greenish appearance as compared with the clear golden yellow tint of normal sera. Another group of affected sera have the appearance of egg-yolk material.

This flocculation can to a certain extent be obviated by using antigens made up with physiological saline alone, but if as little as 0·2 per cent. phenol is added to the saline used for washing off the growths of the oganisms for antigen purposes, flocculation or cloudy reactions occur frequently during testing.

TABLE 5.

Antigen.	pH.	Amounts of normal NaOH solution added to 4 c.c. antigen.	pH.
S3 Titre against Standard serum. 1 : 600	6·8	— 0·01 c.c. 0·02 c.c. 0·03 c.c.	6·8 8·2 8·4 9·0
S5 Titre against Standard serum. 1 : 600	6·8	— 0·01 c.c. 0·02 c.c. 0·03 c.c.	6·8 8·0 8·2 9·0
S6 Titre against Standard serum.	6·8	— 0·01 c.c. 0·02 c.c. 0·03 c.c.	6·8 8·0 8·4 9·0
P81 Titre against Standard serum. 1 : 800	6·8	— 0·01 c.c. 0·02 c.c. 0·03 c.c.	6·8 8·4 8·6 9·0
5206 Titre against Standard serum. 1 : 800	7·2	— 0·01 c.c. 0·02 c.c. 0·03 c.c.	7·2 8·2 8·4 9·0
7536 Titre against Standard serum. 1 : 1,600	7·6	— 0·01 c.c. 0·02 c.c. 0·03 c.c.	7·6 8·4 8·8 9·2
94 Titre against Standard serum. 1 : 1,600	7·4	— 0·01 c.c. 0·02 c.c. 0·03 c.c.	7·4 8·4 8·6 9·2

Hutyra, Marek and Manninger (1938) make mention of cloudiness and flocculation and quote Casman, Valley and Rettger who adjusted the pH of the antigen to 8·2 to 8·5 by the addition of an alkali and so prevented these phenomena.

This method appeared to be more scientific and to offer better possibilities than the mere addition of a measured amount of NaOH to a given quantity of antigen just prior to use, as it seemed likely that the pH of each antigen might vary. The pH of most antigens was found to vary from 6·8 to 7·6 using phenol red as an indicator with a Lovibond Comparator. By the addition of N/10 NaOH in 0·1 c.c. quantities to 4 c.c. of the antigen, prepared as for testing purposes, one was able to adjust the pH to a point between 8·2 and 8·5. In most cases the addition of 0·02 c.c. of normal NaOH solution per 4 c.c. of antigen was sufficient. When one added 0·3 c.c. or more N/10 NaOH the pH reached 9 or more. Seven examples are given in Table 5.

TABLE 6.

Serum.	NaOH.	1/20	1/40	1/80	1/160	1/320	Results.
C1...........	—	++	++	++	++	+	Positive.
	0·75 per cent.	++	++	++	++	++	,,
	2·00 per cent.	++	++	++	++	+	,,
C2...........	—	++	++	++	+	—	,,
	0·75 per cent.	++	++	++	++	—	,,
	2·0 per cent.	++	++	+	—	—	,,
C3...........	—	++	++	++	+	—	,,
	0·75 per cent.	++	++	++	++	—	,,
	2·0 per cent.	++	++	+	—	—	,,
C61..........	—	++	—	—	—	—	,,
	0·75 per cent.	++	++	—	—	—	,,
	2·0 per cent.	++	—	—	—	—	,,
C89..........	—	—	—	—	—	—	Negative.
	0·75 per cent.	—	—	—	—	—	,,
	2·0 per cent.	—	—	—	—	—	,,
A1...........	—	++	++	+	—	—	Positive.
	0·75 per cent.	++	++	++	—	—	,,
	2·0 per cent.	++	—	—	—	—	,,

The method now in vogue is to add 0·75 per cent. normal NaOH solution to all antigens which agglutinate at from 1:600 to 1:1000 and having a pH of 6·8. Antigens having a reaction of 1:1000 to 1:1600 and over, with a pH of more than 7·2 should receive correspondingly less normal NaOH solution, viz., 0·25 per cent. to 0·5 per cent.

Mallmann (1927) in his work agreed with the findings of Mathews (1926) that the sensitivity of *S. gallinarum* antigen was reduced by the use of alkali. This fact could not be confirmed—it was found that *S. gallinarum* antigen to which 0.75 per cent. normal NaOH had been added, was slightly more sensitive than the same antigen to which no NaOH solution or 2 per cent. of it had been added. This does not seem to be a very important point except when the titre of a serum is low, e.g. positive in a 1:10 dilution. A few examples are given in Table 6.

Antigen *S. gallinarum* 75-Allerton titre	1:600
Controls of *S. gallinarum* with no NaOH solution	1:80
Controls of *S. gallinarum* with 0·75 per cent. NaOH solution	1:80
Controls of *S. gallinarum* with 2 per cent. NaOH solution	1:40
Negative controls of all—negative.	

NaOH solution affects the density of the antigen to be used for testing. If one wishes to make up an antigen having an opacity of tube 4 it should first be made up to tube 5 density and then the NaOH solution should be added.

Summary.—(1) Cloudy reactions and flocculation occur mainly from December to the end of May, which is the rainy season; during this period the growth of grass is good and the fowls are in full production.

(2) The addition of phenol in small concentrations has some effect in producing the two phenomena.

(3) The pH of antigens prepared for testing purposes varies from 6·8 to 7·6.

(4) A pH of from 8·2 to 8·5 appears to be the desirable one for combating cloudy reactions and flocculation.

(5) The amounts of normal NaOH solution that are added to antigens for testing purposes should be regulated according to the pH of the antigen as prepared for testing purposes.

(6) Both *S. pullorum and S. gallinarum* antigens are made slightly more sensitive when brought to a pH of 8·2 to 8·5 by the addition of normal NaOH solution.

(7) The addition of NaOH solution should take place after the antigen has been adjusted to the correct density and just prior to its use for testing purposes.

The Positive Reactor to the Single Tube Test.

In reading through literature of work carried out in bacillary white diarrhoea testing in America, it was noted that different workers adopted different dilutions of sera and antigen for the identification of positive reactors to the tube test.

Doyle (1925) when testing experimental birds always commenced with a 1:5 dilution and continued upwards. From the results he obtained it appeared that fluctuation in titre was an important factor when dealing with low-titred birds.

Graham and Tunnicliff (1927) carried out agglutination tests on birds in three dilutions, viz. 1:10, 1:50, and 1:100 As a result of their findings they adopted the 1:10 dilution in all their routine work for the diagnosis of positive reactors.

When the test was first commenced in South Africa complete agglutination in a dilution of 1:20 was interpreted as an indication of a positive reactor. This was adhered to for some time. In the course of post-mortem examinations of birds, giving a partial agglutination in a dilution of 1:20 but a complete agglutination in a dilution of 1:10 after sub-checking the serum, it was surprising how often *S. pullorum* or *S. gallinarum* was isolated from them.

It was suggested that actual carriers were being missed by using a dilution of 1:20, and it was thought that if a flock was tested in a dilution of 1:10 more reactors would be picked out in a shorter time, thus reducing the numbers of rounds of tests necessary to clear up a flock. It was then decided to adopt the dilution of 1:10 as a standard titre.

Results are given of birds showing a trace of agglutination in a dilution of 1:20, but complete in 1:10, which were killed, and on which careful bacteriological examinations were conducted. All these birds were picked out as positive to a dilution of 1:10 and then their sera were titrated up to a dilution of 1:320. In this way the trace of agglutination in a dilution of 1:20 was discovered.

Hen 150: Trace of agglutination in 1:20, complete in 1:10; ovaries dormant, spleen slightly enlarged, whitish yellow pin-point areas under capsule—cultures positive for *S. pullorum.*

Hen C.8841: Trace of agglutination in 1:20, complete in 1:10; marked oophoritis, some ova containing semi-hard yellowish material—cultures from ovaries positive for *S pullorum.*

Hen C.6235: Trace of agglutination in 1:20, complete in 1:10—two big ova filled with semi-solid greenish material; cultures from ovaries positive for *S. pullorum.*

Hen H.3654: Some deformed and shrunken ova containing dirty greyish coloured material; trace of agglutination in 1:20, complete in 1:10—cultures from ovaries positive for *S. pullorum.*

Hen 5003: Trace of agglutination 1:10; ovaries apparently normal; cultures from ovaries positive for *S. pullorum.*

Pullet 432: Trace of agglutination 1:10; cultures from bile positive for *S. gallinarum.*

Hen 7181: Complete agglutination 1:10; cultures from bile positive for *S gallinarum.*

Hen 60: Trace of agglutination in 1:20, complete in 1:10; cultures from ovaries positive for *S. pullorum.*

Hen 13: Trace of agglutination in 1:20, complete in 1:10; cultures from spleen positive for *S. typhi-murium.*

Hen 4250: Trace of agglutination 1:20, complete in 1:10; cultures from ovaries positive for *S. pullorum*.

Hen 4291: Trace of agglutination 1:20, complete in 1:10; cultures from ovaries positive for *S. pullorum*.

*Hen L.R.*53: Trace of agglutination 1:20, complete in 1:10; cultures from bile positive for *S. pullorum*.

It is readily admitted that a large number of birds, giving a trace of agglutination in a 1:20 dilution, give no growth on bacteriological examination, or a growth of organisms other than members of the Salmonella group. It must be stressed that failure to confirm tube test findings may be due to the presence of only a light infection in a single organ of the body, or even to defects in the technique of attempted isolation of the causative organism. Pallaske and Lommatzch (1933) stress a point that has been experienced in this work on *S. pullorum* and *S. gallinarum*, namely, that a number of attempted isolations of *S. enteritidis* (Dublin) cultures after eighteen hours incubation showed no growth, but at 40 hours a growth of the causal organisms was present.

These low titred reactors, if left in a flock, are able to lay infected eggs and could easily form a commencing point for an outbreak of either bacillary white diarrhoea or fowl typhoid at some later date.

It should be understood that a whole flock of fowls is never condemned as the result of picking out a few birds giving a trace of agglutination in a dilution of 1:20. They are obtained from the owner and a careful bacteriological examination is carried out on them. Only on the results of this investigation is a final decision eventually given.

The titres of the sera of positive reactors vary greatly. A list of examples is given. These birds were picked out as giving a trace of, or complete agglutination in a dilution of 1:10. The sera were then titred out in various dilutions from 1:10 to 1:320. These results are typical of all positive reactors. The majority of these low titred birds were examined bacteriologically and the positive test diagnosis confirmed by the isolation of either *S. pullorum* or *S. gallinarum*.

From these figures it will be seen that if the test dilution had been higher than 1:20 a number of reactors would have been missed. (Table 7.)

The next point to be considered is the actual fluctuation in the titres of individual positive birds tested over a period. Examples are given in Table 8.

The figures given are representative of many other fowls examined, and once again it is evident that if high dilutions of sera and antigen are used, numbers of positive reactors are likely to be missed. These figures also bear out the conclusions of Doyle (1925), Newsom, Floyd Cross and Ufford (1928), Greaves, Dearstyne and Gauger (1935), and Edington (1937). Beach, Halpin and Lampman (1927) were of the opinion that the agglutination test was not accurate, and stated in their definition of a reactor that it was a bird whose blood-serum agglutinates the antigen in a dilution of 1:50 or higher.

The variations in titre in individual birds, as shown in the preceding table, indicate clearly what fluctuations may take place.

TABLE 7.

Variations in titres of different fowls.

Fowl.	1–10.	1–20.	1–40.	1–80.	1–160.	1–320.
50 A.	++	–	–	–	–	–
59 A.	++	++	+	–	–	–
68 M.	++	++	++	–	–	–
79 M.	++	++	–	–	–	–
24 B.	++	++	++	++	–	–
83 B.	++	++	++	+	–	–
31 ST.	++	++	++	++	++	–
33 ST.	++	++	–	–	–	–
80 B.	++	++	+	–	–	–
82 B.	++	–	–	–	–	–
13 W.	++	+	–	–	–	–
59 W.	++	++	++	+	–	–
57 W.	++	–	–	–	–	–
62 S.	++	++	++	++	+	–
16 S.	++	+	–	–	–	–
12 E.	++	++	++	++	++	–
86 E.	++	++	++	+	–	–
40 E.	++	++	–	–	–	–
72 A.	++	++	++	++	++	+
98 L.	++	++	++	++	++	+
85 E.	++	++	++	++	++	++
19 C.	++	++	++	–	–	–
77 C.	++	++	+	–	–	–
46 C.	++	++	++	++	–	–
39 F.	++	++	–	–	–	–
22 F.	++	+	–	–	–	–
67 F.	++	++	++	++	++	–
64 F.	++	++	–	–	–	–
76 C.	++	++	+	–	–	–
31 C.	++	+	–	–	–	–
17 M.	+	–	–	–	–	–
41 M.	+	–	–	–	–	–
62 M.	++	–	–	–	–	–
64 P.	++	+	–	–	–	–

+ = trace of agglutination. ++ = complete agglutination.

Summary.—(1) Various laboratories, carrying out the agglutination test of fowl sera for the presence of either *S. pullorum* or *S. gallinarum* carriers rely on different titres of serum and antigen for the identification of positive reactors.

(2) The United States Livestock Sanitary Association supported the adoption of a standard dilution of serum and antigen of 1:25 for picking out carrier birds.

(3) In South Africa a positive reaction, in a dilution of 1:10, is regarded as indicative of a bird that is a carrier of either *S. pullorum* or *S. gallinarum*.

(4) Examples are given of bacteriological examinations carried out on birds showing a trace of agglutination in a 1:20 dilution or complete agglutination in a 1:10 dilution.

(5) Titrations of sera of different fowls show how marked the variations are in the end-points of agglutination.

TABLE 8.

Bird.	22/8/39	18/1/40.	1/2/40.	15/2/40.	29/2/40.	4/4/40.	23/5/40.	18/6/40.	20/7/40.	15/9/40.	18/10/40.	19/11/40.	20/12/40.	8/2/41.	10/3/41
788......	+10	+20	++80	+80	++80	+40	++40								
24.......	+160	+80	++160	+80	++80										
488......	+160	+40	+20	+40	++20	+80	+40								
79.......	+80	++80	++40	+40	++20	+40	++20	+80	+80	+40					
57.......	+160	++160	++160	+80	+160	++20	+40	++40	++80	++80					
196......	++80	+40	++80	+80	++40	+40	++20	++20	++20	++20	++20	++20			
44.......	++10	+20	+10	++20	+40	+20	+80	+40	++20	++20	++20	++20			
15.......	++20	++40	+20	+40	+20	++20	+40	++20	+80	++40	++10	+80	++40		
27.......	+40	+20	++10	++20	+80	+80	++80	+40							
352......	+160	+160	+80	++80	++80	+40	+80	++20	++40	+80	+40	+80			
67.......	++80	+160	+80	+80	+160	++40	++80								
67.......	++80	+160	+80	+80	+160	++40	++80								
4014.....	+40	+40	+40	+160	+80	++160	++80	+80	++40	++80	++160	++160	++20	++40	++40
4024.....	+160	+80	++40	++80	++80	+80	++80	++80	+80	++40	++40	+80	+20		
4045.....	+10	++10	+10	+40	++10	++10	++10	++40	+80	+10	+20	++10			
4064.....	+40	+20	+10	++20	+20	+160	++160	+40	++40	++40	++80	++40	+40	++10	++20
4067.....	++40	+80	+80	+160	++80	+80	++160	++80	++160	+160	++80	++160	++40		
4089.....	++40	+40	++20	+40	+80	+40	+80	+40	++40	+80	+40	+40	+40		
4092.....	++80	++40	++80	+40	++40	++20	+80	++80	++40	++40	++40				
4203.....	+40	+80	+80	+40	++40	++80	+80	++80	++160	++80	+40	+80	++40		
4205.....	++40	++10	++10	++40	++80	++80	+80	+$\frac{3}{8}$	+80	+40	+80	++20	++40		
4233.....	++40	++20	+80	++40	++20	+10	++40	+20	++40	++20	+40				
4235.....	+80	++10	+20	+40	+20	+20	++20	++40	+20						

+ = Trace of agglutination. ++ = Complete agglutination.

(6) Marked fluctuations are shown in the positive titre of individual fowls tested over a period of time.

Intermittent Reactions of Fowls to the Agglutination Test.

Greaves, Dearstyne and Gauger (1935) quote work done by Beach (1927), Edwards and Hull (1929), Dearstyne, Kaupp and Wilfong (1929), Dearstyne, Greaves and Gauger (1931) and Dalling and Warrack (1930) attesting to this phenomenon of intermittency of reaction. The suggestion by Dalling and Warrack (1930) that intermittent reactors were birds that were poor agglutinin producers and only produced them at certain periods of their lives, e.g. when in full lay, is a very reasonable one. That such cases may occur is not questioned but, until other factors are examined, such as antigens used in tests, methods of carrying out tests, standardization of antigens, and test dilutions indicating positive reactors, etc., it is difficult to believe that many such birds exist.

Greaves, Dearstyne and Gauger (1935) claimed that most of the intermittent reactors studied were typically low titred birds, their lowest positive dilution being 1:25. On perusing the tabulated list of individual birds showing fluctuations in titre over a period of time, it will be seen that a number of these birds might be classed as intermittent reactors, e.g., birds 488, 44, 15, 27, 4045, 4205, and 4235. However, it will be noticed that all these low titres occurred on one date, viz., 1/2/40. It might be suggested that the titre of the antigen had fallen, perhaps due to age, as most of the titres were lower or the same as at testing on 22/8/39.

When the question of continuous intermittency, over a period of time, is considered, the following birds at random may be quoted (Table 9):—

TABLE 9.

Bird.	22/8/39	18/1/40	1/2/40	15/2/40	29/2/40	4/4/40	23/5/40	18/6/40	20/7/40	15/9/40	18/10/40	19/11/40
44......	++10	+20	+10	++20	+40	+20	+80	+40	++20	++20	+20	++20
15......	++20	++40	+20	+40	+20	++20	+40	++20	+80	++40	++10	+80
4045....	+10	++10	+10	+40	++10	++10	++10	++40	+80	+10	+20	++10
4235....	+80	++10	+20	+40	+20	+20	++20	++40	+20			
4280....			+10	+10	N.	+10	++20					

\+ = Trace of agglutination. ++ = Complete agglutination.

All these birds, on bacteriological examination, yielded cultures of either *S. pullorum* or *S. gallinarum*. These five birds are good examples of poor agglutinin producers.

A further explanation in the intermittent reactor phenomenon may be the fact that single strain antigens, as compared with antigens made up of a number of strains, will usually detect a larger number of reactors. In addition, in certain flocks an antigen showing a low standard value will detect more reactors than an antigen showing a much higher standard value.

Finally, it does not follow that antigens composed of several strains, and having a high standard value, will always give a higher positive agglutination with any one positive fowl serum than antigen of a lower standard titre.

A table of comparison is given. (Table 10.)

TABLE 10.

Fowl.	Titre of fowl sera against Antigen 67 (*Pullorum*) Allerton Standard 1 : 1,000.	Titre of fowl sera against Antigen 69 (*Pullorum*) Allerton Standard 1 : 800.	Titre of fowl sera against Antigen 68 (*Pullorum*) Allerton Standard 1 : 600.
11	Complete 1 : 320	Trace 1 : 320	Complete 1 : 160
13	Trace 1 : 40	Trace 1 : 40	Trace 1 : 20
14	Complete 1 : 320	Trace 1 : 40	Trace 1 : 320
24	Trace 1 : 320	Trace 1 : 160	Complete 1 : 80
27	Trace 1 : 320	Complete 1 : 160	Trace 1 : 160
32	Trace 1 : 20	Negative.	Negative.
39	Complete 1 : 320	Complete 1 : 160	Complete 1 : 320
41	Trace 1 : 160	Trace 1 : 80	Complete 1 : 40
45	Complete 1 : 40	Trace 1 : 40	Trace 1 : 40
46	Complete 1 : 320	Trace 1 : 80	Complete 1 : 80
50	Trace 1 : 80	Trace 1 : 20	Trace 1 : 40
54	Negative.	Negative.	Negative.
56	Trace 1 : 160	Trace 1 : 80	Trace 1 : 80
65	Trace 1 : 20	Negative.	Trace 1 : 20
66	Complete 1 : 320	Trace 1 : 160	Trace 1 : 160
67	Trace 1 : 20	Negative.	Negative.
78	Trace 1 : 80	Trace 1 : 40	Trace 1 : 40
Control positive.	Trace 1 : 160	Complete 1 : 80	Complete 1 : 80
Control negative.	Negative.	Negative.	Negative.

Summary.—(1) It is stated by a number of overseas workers that there are birds which give only intermittent reactions to the agglutination test.

(2) The suggestion has been made that such birds are poor agglutinin producers and that these agglutinins are produced only during certain periods of their lives.

(3) It is considered that in many such cases not enough stress has been laid on antigens used for testing, value of antigen used, and the dilutions of the tests that indicate reactors.

(4) An important point to consider is the normal fluctuations in positive titres that regularly occur in the majority of carriers of *S. pullorum* or *S. gallinarum* infection.

(5) In certain flocks an antigen showing a low standard value will pick out more reactors than will an antigen of a much higher standard.

(6) It does not always follow that antigens composed of several strains of the organisms, and having a high standard value, will always give a higher positive titre with any one known positive fowl serum than an antigen of a lower standard titre.

The Comparison of Titres of Positive Fowl Sera when Tested with S. pullorum *and* S. gallinarum *Antigens.*

It is logical to reason that in testing for the presence of bacillary white diarrhoea an antigen made up of a strain or strains of *S. pullorum* should be used, and if searching for fowl typhoid carriers an antigen of *S. gallinarum* should be utilized. Continuing this deduction, an antigen composed of strains of *S. gallinarum* and *S. pullorum* should pick out either fowl typhoid or bacillary white diarrhoea at the one test.

That most workers preferred a *S. pullorum* antigen is understandable, as such an antigen appears to retain its powers of agglutination better than does an *S. gallinarum* antigen. Where antigens are made frequently and used within a short time of their preparation, as is the case in laboratories where large numbers of tests are carried out, a fresh *S. gallinarum* antigen, if used for testing purposes, will pick out as many reactors as will a *S. pullorum* antigen. Furthermore, a much larger harvest of antigen will be obtained when growing *S. gallinarum*, instead of *S. pullorum*, in the same number of Roux and Fernbach flasks. When antigen composed of *S. pullorum* and *S. gallinarum* strains is used in comparative tests against antigens made of *S. pullorum* or *S. gallinarum*, very little, if any, difference is observed in the number of positive reactors picked out by any of them. Among the positive reactors picked out by any of the three types of antigens there will be either bacillary white diarrhoea or fowl typhoid carriers; consequently no advantage is gained by using an antigen made up of both *S. gallinarum* and *S. pullorum* strains. In fact, practically identical results will be obtained whether the antigen is made up of either *S. pullorum* or *S. gallinarum*.

The statement by Mathews (1926), that when any fowl serum was tested the positive reaction of the *S. pullorum* antigen was always higher than that of a *S. gallinarum* antigen, could not be confirmed. In some cases the *S. gallinarum* antigen gave a higher positive reaction while in others the *S. pullorum* antigen yielded higher ones. A number of such cases are given in the form of tables 11, 12, 13 and 14. These figures are taken from a large number of comparisons carried out and bear out the statement that there are no great variations between *S. pullorum* and *S. gallinarum* antigens.

Variations often occur when sera are tested against a number of different antigens, all of them prepared from *S. gallinarum*. A *S. pullorum* antigen was added for the sake of comparison.

TABLE 11.

Fowl.	Titre against *S. pullorum* antigen 37.	Titre against *S. gallinarum* antigen 66.	
6	Trace in 1 : 160	Trace in 1 : 320	Allerton Standard of *Pullorum* 37—a trace in 1 : 720.
15	Trace in 1 : 80	Trace in 1 : 160	
44	Trace in 1 : 160	Trace in 1 : 320	Allerton Standard of *Gallinarum* 66—a trace in 1 : 800.
68	Trace in 1 : 320	Trace in 1 : 320	
68A	Trace in 1 : 40	Trace in 1: 40	Titre of *Pullorum* 37 against positive control fowl serum—complete in 1 : 160.
97	Trace in 1 : 160	Trace in 1 : 160	
23	Trace in 1 : 80	Trace in 1 : 80	Titre of *Gallinarum* 66 against positive control fowl serum—trace in 1 : 320.
27	Complete in 1 : 160	Trace in 1 : 160	
41	Trace in 1 : 160	Trace in 1 : 160	
20	Trace in 1 : 80	Trace in 1 : 160	
29	Trace in 1 : 80	Complete in 1 : 80	
36	Trace in 1 : 20	Trace in 1 : 20	
38	Trace in 1 : 160	Complete in 1 : 160	
54	Complete in 1 : 80	Complete in 1 : 160	

TABLE 12.

Fowl.	Titre against *S. pullorum* antigen 38.	Titre against *S. gallinarum* antigen 67.	
1	Trace in 1 : 80	Trace in 1 : 80	Allerton Standard of *Pullorum* 38—a trace in 1 : 720.
13	Complete in 1 : 40	Trace in 1 : 40	
14	Trace in 1 : 80	Trace in 1 : 40	
19	Trace in 1 : 40	Trace in 1 : 20	
23	Trace in 1 : 160	Trace in 1 : 80	Allerton Standard of *Gallinarum* 67—a trace in 1 : 720.
27	Trace in 1 : 40	Complete in 1 : 20	
33	Trace in 1 : 80	Trace in 1 : 40	
38	Trace in 1 : 160	Trace in 1 : 80	
41	Trace in 1 : 80	Complete in 1 : 20	Titre of *Pullorum* 38 against positive control fowl serum—complete in 1 : 80.
42	Complete in 1 : 40	Trace in 1 : 40	
43	Trace in 1 : 160	Trace in 1 : 40	
45	Trace in 1 : 80	Complete in 1 : 20	
51	Trace in 1 : 40	Trace in 1 : 40	
52	Trace in 1 : 80	Complete in 1 : 40	Titre of *Gallinarum* 67 against positive control fowl serum—trace in 1 : 80.
60	Complete in 1 : 20	Negative.	
70	Trace in 1 : 40	Complete in 1 : 20	
75	Trace in 1 : 20	Trace in 1 : 20	
90	Trace in 1 : 40	Trace in 1 : 20	
92	Complete in 1 : 20	Complete in 1 : 20	
95	Complete in 1 : 20	Negative.	

TABLE 13.

Fowl.	Titre against *S. gallinarum* antigen 58.	Titre against *S. gallinarum* antigen 60.	Titre against *S. gallinarum* antigen 61.	Titre against *S. pullorum* antigen 35.
48.........	Negative.	Negative.	Negative.	Complete in 1 : 20.
78.........	Trace in 1 : 20	Negative.	Negative.	Negative.
1533.......	Trace in 1 : 20	Negative.	Negative.	Negative.
43.........	Complete in 1 : 20	Negative.	Negative.	Negative.
41.........	Trace in 1 : 20	Negative.	Negative.	Negative.
47.........	Trace in 1 : 80	Complete in 1 : 20	Trace in 1 : 40	Trace in 1 : 80
919........	Trace in 1 : 80	Trace in 1 : 20	Complete in 1 : 20	Complete in 1 : 40
129........	Complete in 1 : 20	Trace in 1 : 20	Trace in 1 : 20	Complete in 1 : 20
67.........	Trace in 1 : 40	Trace in 1 : 20	Negative.	Complete in 1 : 20
24.........	Trace in 1 : 20	Negative.	Negative.	Complete in 1 : 20
79.........	Trace in 1 : 40	Negative.	Negative.	Trace in 1 : 20
3..........	Complete in 1 : 20	Negative.	Negative.	
11.........	Trace in 1 : 40	Negative.	Negative.	
13.........	Complete in 1 : 80	Trace in 1 : 80	Trace in 1 : 80	
17.........	Trace in 1 : 320	Trace in 1 : 40	Trace i n 1 : 40	
20.........	Trace in 1 : 80	Complete in 1 : 20	Trace in 1 : 40	
21.........	Trace in 1 : 160	Trace in 1 : 40	Trace in 1 : 80	
23.........	Trace in 1 : 160	Trace in 1 : 20	Complete in 1 : 20	

Allerton Standard of *Gallinarum* 58—complete in 1:1600
Allerton Standard of *Gallinarum* 60—complete in 1:1000.
Allerton Standard of *Gallinarum* 61—complete in 1:800.
Allerton Standard of *Pullorum* 35—trace in 1:720.

Finally, examples can be given of the variations that occur when a serum is titrated against various *Pullorum* antigens. It will be seen that these differences are not confined to *Gallinarum* antigens.

TABLE 14.

Fowl.	Titre against *S. pullorum* antigen 67.	Titre against *S. pullorum* antigen 68.	Titre against *S. pullorum* antigen 69.
11.............	Complete in 1 : 320	Complete in 1 : 160	Trace in 1 : 320
13.............	Trace in 1 : 40	Trace in 1 : 20	Trace in 1 : 20
14.............	Complete in 1 : 320	Trace in 1 : 320	Trace in 1 : 40
24.............	Trace in 1 : 320	Complete in 1 : 80	Trace in 1 : 160
27.............	Trace in 1 : 320	Trace in 1 : 160	Complete in 1 : 160
32.............	Trace in 1 : 20	Negative.	Negative.
39.............	Complete in 1 : 320	Complete in 1 : 320	Complete in 1 : 160
41.............	Trace in 1 : 160	Complete in 1 : 40	Trace in 1 : 80
45.............	Complete in 1 : 40	Trace in 1 : 40	Trace in 1 : 40
46.............	Complete in 1 : 320	Complete in 1 : 80	Trace in 1 : 80
50.............	Trace in 1 : 80	Trace in 1 : 40	Trace in 1 : 20
54.............	Negative.	Negative.	Negative.
56.............	Trace in 1 : 160	Trace in 1 : 80	Trace in 1 : 80
65.............	Trace in 1 : 20	Trace in 1 : 20	Negative.
66.............	Complete in 1 : 320	Trace in 1 : 160	Trace in 1 : 160
67.............	Trace in 1 : 20	Negative.	Negative.
78.............	Trace in 1 : 80	Trace in 1 : 40	Trace in 1 : 40

Allerton Standard of *Pullorum* 67—trace in 1:1000.
Allerton Standard of *Pullorum* 68—trace in 1:600.
Allerton Standard of *Pullorum* 69—trace in 1:800.

From the examples given and from the figures of many other tests carried out, it would seem impossible to distinguish between *S. pullorum* and *S. gallinarum* solely on the results of the comparative titrations of various positive fowl sera.

However, these variations are really only of academic interest. The main point brought out is that, even if the positive test dilution is as low as 1:10, every antigen will not always pick out all the same positive reactors. It has been found that if a certain antigen has missed a positive reactor, by the time the following round has been completed using a different antigen or at the latest, the one after, this positive will have been found. This is a strong argument for insisting on two negative tests with an interval of twenty-one or thirty days between them. It is also a further explanation of why all positives are not picked out by one round of testing.

Summary.—(1) There is little, if any, difference in test results when using either a *S. pullorum* or a *S. gallinarum* antigen, provided the *S. gallinarum* antigen is not old.

(2) Both *S. pullorum* and *S. gallinarum* antigens will pick out either bacillary white diarrhoea or fowl typhoid carriers; therefore nothing is to be gained by using an antigen made up of a mixture of *S. gallinarum* and *S. pullorum* strains.

(3) The claim that *S. pullorum* can be differentiated from *S. gallinarum*, as the result of a higher positive titre in the case of *S. pullorum*, could not be confirmed.

(4) Differences in positive titre, in some cases marked, will be observed when submitting the same sample of serum to different batches of either *S. gallinarum* antigens or *S. pullorum* antigens. They may be observed when a sample of serum is tested by a *S. pullorum* antigen and sub-checked by a *S. gallinarum* antigen or vice versa.

(5) Most of these variations are merely of academic interest and not of practical importance. except that they further stress the importance of using a small dilution in picking out reactors.

(6) It is admitted that all reactors will not be picked out by every antigen used, and therefore all positives are unlikely to be found merely by one round of testing.

The Positive Reaction to the Agglutination Test.

The tube showing a typical positive reaction cannot be mistaken. The fluid is, if possible, more than water clear; it takes on an appearance that could be compared with the brightness of a clean mirror. At the bottom of the tube is a fluffy mass of agglutinated bacilli, and down the length of the tube will usually be found very small specks of similar cottonwool-like masses, which are composed of agglutinated organisms that have adhered to the side of the tube.

A second type of positive reaction will show a mirror-like clearness with no small accumulations down the side of the tube.

A third type of reaction is sometimes seen, and this is difficult to interpret. The supernatant fluid is clear and at the bottom of the tube is a mass of organisms. This mass shows a closely packed distinct level surface. There are no small white collections of organisms adherent to the

sides of the tube and the supernatant liquid has not the mirror-like clearness seen in the definite positive reaction. In different cases all gradations in opacity, from the clear reaction to a complete negative, can be observed. This reaction seems to be a sedimentation or a spontaneous agglutination rather than a true agglutination.

It would be impossible to state that all reactions of such type are negative, but in many cases they cannot be confirmed by re-testing with different *S. pullorum* or *S. gallinarum* antigens or even by a bacteriological examination of the fowl.

It has been found in some cases that the use of dirty tubes for the test, or even of a dirty burette for filling tubes with antigen, may give rise to such reactions. A common cause of this condition is an infection of the fowl with organisms other than members of the Salmonella group.

Hinshaw and Dunlop (1928) drew attention to what they termed "partial" reactions and described the condition, which undoubtedly is the third type of reaction mentioned above. They showed that these reactions could be caused by a Gram-positive coccus that was found in the agglutination tubes at the time these pseudo-positive reactions took place.

Hurt and McCulloch (1940) reported the isolation of a Gram-positive coccus from chickens and adult birds that gave a positive reaction to the test, but also in some cases from chickens that did not react. This organism was a lactose fermenter.

In the course of sub-checking these false positive reactions our experience has been that cocci are frequently isolated in pure culture from the ovaries, but in addition, a further group of organisms has been encountered. These are Gram-negative rod-shaped organisms that do not give rise to acid with lactose but ferment maltose, glucose and saccharose. On brilliant green agar their growth resembled a typical *S. pullorum* growth.

A number of these Gram-negative bacilli and cocci were cultivated and antigens prepared from them. They were then tested against known positive and negative fowl serum. (See Table 15.)

TABLE 15.

Bird.	Positive Serum.					Negative Serum.				
	1/10	1/20	1/40	1/80	1/160	1/10	1/20	1/40	1/80	1/160
5109	++	+	–	–	–	++	++	–	–	–
9314	++	++	++	++	+	++	++	+	+	+
335	++	++	++	+	–	++	++	–	–	–
104	++	++	+	–	–	++	++	+	–	–
430	++	+	–	–	–	+	+	–	–	–
3219	+	+	+	+	–	+	+	+	+	–
9247	++	+	++	++	–	++	++	++	++	++

Positive serum with Antigen P63 = +80.
Negative serum with Antigen P63 = negative.

Post-mortem reports on these birds are as follows:—

5109—apparently normal, no lesions.

9314—slight catarrhal enteritis.

335—very slight oophoritis, two small ova of dirty yellowish colour, rest normal.

104—ovaries apparently normal, bird very fat.

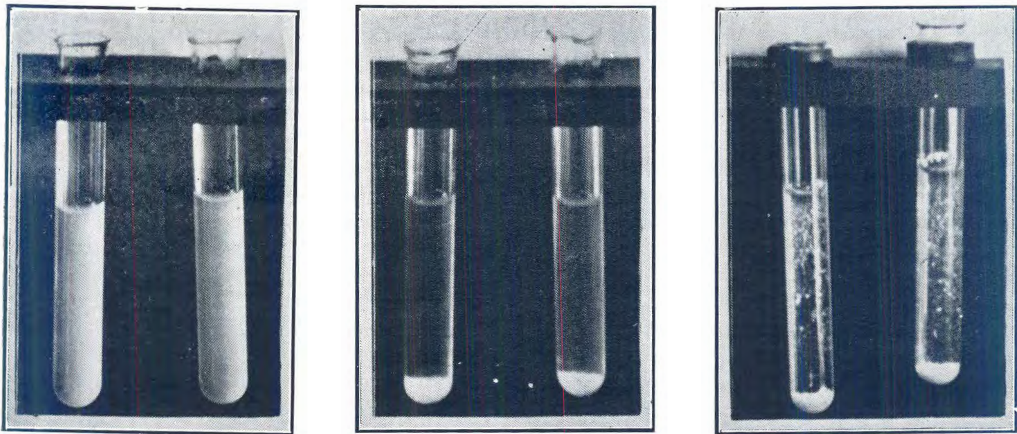
430—ovaries apparently normal, bird very fat.

3219—ovaries apparently normal, Mullerian cyst. Agglutination test with liquid from cyst negative. Cultures from cyst—no growth.

9247—one shrunken ovum of greenish colour, rest normal.

No organisms of the Salmonella group were obtained from any of these birds.

A photograph is given showing a negative, a positive and a false positive reaction.



Negative reaction. False positive reaction. Positive reaction.

Fig. 8.

Summary.—(1) Descriptions of two varieties of positive reactions are given.

(2) A description of a false positive reaction is given.

(3) Contamination of tubes and testing apparatus does sometimes set up this false positive reaction.

(4) This reaction should be controlled by re-testing such sera samples.

(5) The more common cause of this false positive reaction is the presence in either the ovaries or bloodstream of the fowl of Gram-negative bacilli or Gram-positive cocci.

(6) Most of these affected birds show this false positive reaction only in low dilutions of the test.

(7) These organisms may or may not give rise to acid with lactose, but some ferment saccharose.

(8) Such birds show few, if any, lesions of the ovaries.

(9) This infection does not appear to cause any disease in the carrier.

The Age of Birds when Testing may be Commenced.

The question has often been asked " At what age can testing be commenced? ". Varying opinions have been expressed.

Rice (1930) was of the opinion that there was no agreed age at which birds should be tested, but suggested that the best time would appear to be about two months after the birds began to lay. His reasons were that at this stage the causal organism had probably become localised in its predilection seat, the functioning ovary, or the pullets had lost the agglutinins engendered by infection, which had failed to establish itself.

It has been the experience here that many chickens under four months do not react as a rule and probably will not react until they have reached maturity. This at times has caused a certain amount of disappointment among farmers who have been submitting their birds to testing.

An outbreak of bacillary white diarrhoea in day-old chicks took place in the vicinity of the Laboratory. This was confirmed by bacteriological examination. After a few days the mortality stopped. The owner kept two hundred of this batch, which were isolated, and the rest were killed. Just prior to the first pullet laying, a blood test was done on them and one positive reactor was found. *S. pullorum* was isolated from the heart blood. A month later, when most of them had commenced laying, a second test was carried out when twenty odd birds gave positive reactions to the agglutination test; in addition some died and from them *S. pullorum* was isolated. The owner presented the twenty positive birds to the Laboratory and they have given positive reactions ever since; the remaineder were sold as dressed poultry.

There is still another aspect to consider. Three breeds of chickens, in groups of twenty-five, from a badly infected farm, were received at the Laboratory when they were two days old. Within fourteen days 99 per cent. of the Rhode Island Red birds died and from them *S. pullorum* was isolated. Odd deaths took place among the Australorps and again *S. pullorum* was recovered. All but two of the White Leghorns survived and from these two birds *S. pullorum* was cultivated. At three months of age all were blood tested and ten of the birds gave complete agglutinations in dilutions of 1:10. Some of these birds gave positive agglutination in titres up to 1:40, 1:80 and one incomplete agglutination in 1:160. A number gave negative results.

They were tested either every two weeks or monthly up to the age of ten months, when only one bird maintained a titre of 1:40. The remainder have now given a number of negative tests or incomplete agglutinations in a 1:10 dilution. (see Table 16). Van Roekel (1932) also stresses this point.

TABLE 16.

Consecutive Tests of Chickens from badly infected Farm.

No.	5/11/43.	23/11/43.	6/12/43.	17/12/43.	5/1/44.	20/1/44.	8/2/44.	1/3/44.	20/4/44.	16/6/44.	
1......	—	—	—	—	—	—	—	—		—	
2......	—	—	—	—	—	—	—	—		—	
3......	—	—	—	—	—	—	—	—		—	
4......	—	—	—	—	—	—	—	—		—	
5......	—	—	—	—	—	—	—	—	—	—	
6......	—	—	—	—	—	—	—	—		—	
7......	—	—	—	—	—	—	—	—		—	
8......	++20	—	+10	++10	++10	—	—	—	—	—	
9......	—	—	—	—	—	—	—	—		—	
10......	—	—	—	—	—	—	—	—		—	
11......	—	—	—	—	—	—	—	—		—	
12......	—	—	—	—	—	—	—	—		—	
13......	—	—	—	—	—	—	—	—		—	
14......	—	—	—	—	—	—	—	—		—	
15......	—	—	—	—	—	—	—	—		—	
16......	—	—	—	—	—	—	—	—		—	
17......	++20	—	—	—	—	—	—	++20			Died 16/3/44. Nothing isolated.
18......	++10	++20	++20	++20	++10	+20	+40	++40	+80	+40	
19......	—	—	—	—	—	—	—	—	—	—	
20......	++40										Died 9/11/43. *S. pullorum* isolated
21......	++40	+10	++10	++10	+10	—	—	—	—	—	
22......	+20	++10	++20	+40	++10	+10	+10	—			Died 18/3/44. Nothing isolated.
23......	—	—	—	—	—	—	—	—			Died 18/3/44. Nothing isolated.
24......	+20	++10	++20	+40	++10	—	+20	++10	+10	+10	
25......	—	—	+10	—	—	—	—	—	—	—	
26......	+160	+40	+40	++20	++10	—	—	—	++10	—	
27......	+80	+10	+20	++10	—	—	+10	—	—	—	
28......	—	—	+10	—	—	—	+10	—			Died 4/4/44. Aspergillosis.
29......	++10	—	++10	++10	—	—	—	—	—	—	

This would appear to indicate that the ten chickens were infected through the egg, and had a generalised infection which stimulated the production of agglutinins. As time passed no localization took place except in bird No. 18 and the infection died out, with the result that the agglutinins in the blood disappeared. It must, however, be borne in mind, that these young chickens were placed in clean runs where there was no possibility of their acquiring further infection. Two different groups of cases have thus been quoted.

In the first group, had testing been carried out before egg-laying started, a number of reactors would have been missed, and, by the time the following annual test had been done, a large number of infected eggs would have been laid and active infection would have been set up.

In the second group, had testing been done early, a number of birds would have been condemned as positive, which, later, at about five months, had lost the infection, and gave negative tests.

Finally, it has been observed that, when testing on an infected farm pullets that are about to commence laying, or have just started to lay, a number of doubtful reactions to the test are given, both with a *Pullorum* or a *gallinarum* antigen. These are difficult to interpret. Whether, when laying well, they give a distinct positive or distinct negative is a point that needs investigation.

Summary.—(1) Young stock should be tested only after egg-laying has started, or at 5 to 6 months of age.

(2) Many of the doubtful reactions to the test are to be found in young birds just commencing to lay, or that have just started to lay, especially if they are from farms where there have been large numbers of positive reactors.

Conclusions.

(1) Standardization should be carried out with all strains of organisms and antigens prior to utilization for testing purposes. Single strains of organisms should be submitted to low dilution testing before use as antigens.

(2) Known negative fowl serum should be used for negative control tests.

(3) The water of condensation should be poured or siphoned out of flasks of growing cultures before washing off the growths for the preparation of antigen.

(4) *S. pullorum* antigen appears to be a much more stable product than *S. gallinarum* antigen. Reliable results can be expected from *S. pullorum* antigens for at least two years after preparation, but *S. gallinarum* antigens over a year old should be checked prior to use for testing purposes.

(5) The highest titred antigens are obtained by using single strains of *S. pullorum* or *S. gallinarum* for antigen production.

(6) In most cases more positive reactors are picked out in badly infected flocks when single strain antigens are used, in preference to antigens made up of a number of strains.

(7) The higher the titre of the single strain antigen the more positive reactors that are picked out, but one must be careful of so-called supersensitive strains. In a small number of cases a lower titred antigen may pick out more reactors than an antigen with a high titre.

(8) To expedite the eradication of positive reactors from an infected flock it is advisable to use single strain antigens with high values.

(9) The addition of small amounts of phenol has some effect in influencing the phenomena of "cloudy reactions" and flocculation in the tube test. The amount of normal NaOH solution added to antigens to prevent these two phenomena should be regulated by the pH of the antigen. Both *S. pullorum* and *S. gallinarum* antigens are made slightly more sensitive when adjusted to a pH of 8·2 to 8·5 by the addition of normal NaOH solution.

The addition of the normal NaOH solution to the antigen should take place just prior to its use for testing and, bearing in mind that the density of the antigens is affected by this addition, due allowance should be made for this effect.

(10) In South Africa complete agglutination in a dilution of 1:10 of a serum and antigen is regarded as indicative of a positive bacillary white diarrhoea or fowl typhoid carrier.

(11) Titrations of sera of different fowls show how marked the variations are in the end points of agglutination. Marked fluctuations are shown in the positive titre of individual fowls tested over a period of time.

(12) The intermittent reactor should not be regarded entirely as a fowl that produces agglutinins intermittently. Other factors such as the use of antigens of a low standard titre, and the dilution that is considered to indicate a positive reactor, must be borne in mind.

(13) It does not always follow that an antigen having a high standard titre will always give a higher positive titre with any one known positive fowl serum, than an antigen of a lower standard titre.

(14) There is little, if any, difference in test results when using either a *S. pullorum* or a *S. gallinarum* antigen provided the *S. gallinarum* antigen is only a few weeks old. Both *S. pullorum* or *S. gallinarum* antigens will pick out either bacillary white diarrhoea or fowl typhoid carriers; therefore there does not appear to be anything gained by using an antigen made up of a mixture of strains of *S. pullorum* and *S. gallinarum*.

(15) Differences in the positive titre, in some cases marked, will be observed when the same sample of serum is submitted to a group of *S. pullorum* or *S. gallinarum* antigens.

(16) All carriers of either bacillary white diarrhoea or fowl typhoid will not be picked out by every antigen used, and therefore all positives are unlikely to be detected by one round of testing.

(17) False positive reactions may be caused by the use of dirty glassware, but the more common cause is the presence of organisms, not belonging to the Salmonella group, isolated from the ovaries or from the heart blood. Most of these false positive reactions are found in the low test dilutions.

Not all the organisms that set up these false positive reactions give rise to acid with lactose, and some ferment saccharose.

These organisms give rise to no symptoms in the carriers, which appear to be healthy birds.

(18) Young stock should be tested only after egg-laying has commenced, or at five or six months of age.

Young birds from badly infected farms frequently give doubtful positive reactions to the agglutination test.

ACKNOWLEDGMENTS.

I wish to express my great appreciation to my professional colleagues and friends, D. Haig, W. Schatz and V. R. Kaschula, for much help given me willingly at all times. To my laboratory assistants, Miss N. Upton, F. van Vuuren and L. Hill, my thanks and appreciation are tendered for good work and help.

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APPENDIX.

UNION OF SOUTH AFRICA.

DEPARTMENT OF AGRICULTURE AND FORESTRY.

DIVISION OF VETERINARY SERVICES.

CERTIFICATE.

I hereby authorise..

of..to collect specimens of blood from fowls and turkeys for the Bacillary White Diarrhoea Test, in consideration of the fact that he/she has proved to my satisfaction that he/she is capable of collecting the specimens in an approved manner.

This certificate entitles the holder, by private arrangement with the owner of any fowls and turkeys from which specimens of blood are taken, to charge a fee not exceeding one penny for each specimen of blood collected.

This certificate may be revoked at the discretion of the Director of Veterinary Services if, in his opinion, the holder has been negligent or incompetent.

Signed

DIRECTOR OF VETERINARY SERVICES.

Date :..

N.B.—This certificate is not required by a registered veterinarian.

UNIE VAN SUID-AFRIKA.

DEPARTEMENT VAN LANDBOU EN BOSBOU.

AFDELING VEEARTSENYDIENS.

SERTIFIKAAT.

Hiermee bemagtig ek..

van..om bloedmonsters van hoenders en kalkoene vir die Basilêre Wit Diarree Toets te versamel, aangesien hy/sy tot my tevredenheid bewys het dat hy/sy bekwaam is om die monsters op 'n goedgekeurde wyse te versamel.

Deur onderlinge reëling met die eienaar van hoenders of kalkoene waarvan bloedmonsters versamel word, is die houer van hierdie sertifikaat geregtig om 'n fooi te vra van nie meer as een pennie vir elke bloedmonster wat hy versamel.

Hierdie sertifikaat kan deur die Direkteur van Veeartsenydiens herroep word, as die houer daarvan, na sy mening, nalatig of onbekwaam is.

Geteken :

DIREKTEUR VAN VEEARTSENYDIENS.

Datum :..

Let Wel.—Hierdie sertifikaat word nie verlang van 'n geregistreerde veearts nie.

BLEEDER'S CERTIFICATE.

U.A.D. 875 B.

UNION OF SOUTH AFRICA.

DEPARTMENT OF AGRICULTURE AND FORESTRY.

BACILLARY WHITE DIARRHOEA AND FOWL TYPHOID.

I, ...

of (Farm and Number)...

Post Office..District ....

in consideration of all my fowls and turkeys being submitted to tests for Bacillary White Diarrhoea and Fowl Typhoid by the Veterinary Division of the Department of Agriculture and Forestry acting under the direction of the Director of Veterinary Services, do hereby agree :—

1. To place sealed or seamless and differently numbered leg or wing bands on all my fowls and/or turkeys over 4 months old.
2. That specimens of blood from all my fowls and turkeys over 4 months old shall be taken for laboratory test at such reasonable times, in such manner as may be approved, and by a person authorised by the Director of Veterinary Services.
3. That if the result of the first test of blood specimens is negative, I undertake to test all the young untested fowls and turkeys I now have, as they reach the age of 4 months, and in any case by the end of next March.

   Should all the fowls and turkeys, I now have, pass this first test without reactors being revealed, I understand the B.W.D. and Fowl Typhoid test certificate will be granted. I then undertake to forward to the Director similar specimens yearly, or at such times as he may deem necessary, from all of my fowls and turkeys over 4 months old, whether tested previously or not.

   Should reactors to the test be found in any of the fowls or turkeys tested, I undertake to send to the Director whatever blood samples he may require, and whenever he may submit a written request for them, on the understanding that I shall not have to bleed any bird more often than once in four weeks, and that I shall not be called on, in order to obtain the test certificate to have the whole undivided flock of fowls and turkeys pass more than 2 consecutive clean tests.
4. To allow a Government Veterinary Officer access at all reasonable times to any or all of my fowls and turkeys for the purpose of collecting specimens of blood for verification purposes.
5. That all additions to my flock of fowls and/or turkeys shall be isolated, banded (vide Clause 1) and tested when brought on the premises, and, before being added to my flock, such fowls and/or turkeys shall be retested, the retest to take place 30 to 40 days after the first test—provided that the testing of additions to the flock will not be necessary if they come from a flock which, after being tested as provided for in paragraphs 2 and 3 hereof, has been certified to be free from reactors to the B.W.D. and Fowl Typhoid test.

   Any fowls and/or turkeys added to my flock in terms of this paragraph, shall from time to time be subject to such tests as are provided for in paragraphs 2, 3 and 4 hereof.
6. Not to bring fertile hen or turkey eggs, or fowls or turkeys less than 3 months old on to my premises, after signing this agreement, unless such eggs, fowls or turkeys come from B.W.D. and Fowl Typhoid free flocks, or to send hen or turkey eggs to any hatchery not situated on my premises, if the chicks hatched from such eggs are to be returned to me, or such chicks are to be disposed of in my name or in the name of my poultry business, unless the hatchery accepts eggs only from B.W.D. and Fowl Typhoid -free poultry farms.
7. That the fee to be paid by me to the Director of Veterinary Services shall be one penny in respect of each specimen of blood tested by him. (This should not be confused with any fee payable to the person authorised by the Director of Veterinary Services for taking specimens of blood as set out in Clauses 2 and 3 or any registered veterinarian. The latter fee is a matter for arrangement between the owner of the birds and the person taking the specimens).
8. To surrender to a Government Veterinary Officer for destruction or disposal in a manner deemed necessary by him, all fowls and/or turkeys reacting to the aforesaid tests, and not to remove them from my premises without the written consent of a Government Veterinary Officer, it being understood that I shall claim no compensation in respect of any birds so surrendered by me, and destroyed or disposed of by him.
9. To carry out any reasonable advice given by a Government Veterinary Officer for the purpose of preventing the spread of infection on my premises.
10. To refrain from mentioning B.W.D. or Fowl Typhoid or the blood test for them, in any way whatsoever, in the course of communications or advertisements made by me, or on my behalf, regarding the disposal of any of my poultry or eggs, unless I actually hold the B.W.D. and Fowl Typhoid test certificate, or unless I specifically state that I do not possess the test certificate.

11. That should any other Salmonellosis be found in my stock, I shall send blood samples as for the B.W.D. and Fowl Typhoid test, whenever requested by the Director of Veterinary Services to do so. I am willing to send the blood samples from any or all of my poultry, including fowls, turkeys, ducks, geese, domesticated guinea fowls, and pigeons, and I shall surrender all reacting birds revealed by these tests, on the same terms as B.W.D. and Fowl Typhoid reactors. I am, moreover, willing to send the Director of Veterinary Services such samples of blood as he may request, even if he only suspects the presence of any particular Salmonellosis in my fowls or other species of poultry. I understand, in making these promises that the Director of Veterinary Services will waive the charge of one penny per test, when the birds are being tested actually for this other Salmonellosis.
12. To surrender upon demand the B.W.D. and Fowl Typhoid test certificate in the event of (*a*) reactors being found or (*b*) any of the provisions in clauses 1 to 11 being violated by me.
13. To make no claim against the Department of Agriculture and Forestry for any injury or loss sustained by me, as the direct or indirect result of any of the aforesaid tests.

My flock contains.............................fowls and turkeys over 3 months old.

Witness.. Signed..

Date.. Date..

AGREEMENT FORM SIGNED BY APPLICANTS FOR TESTING OF FOWLS FOR B.W.D. OR FOWL TYPHOID.

All correspondence in connection with the Bacillary White Diarrhoea Test must be conducted with the Officer in Charge, Allerton Laboratory, P.O. Box 405, Pietermaritzburg. This form *in triplicate* must accompany all samples of blood. All three forms must be filled in clearly by the bleeder, and be sent in the box with the samples. One copy will be returned by the Laboratory to the owner of the birds. *A cheque, money-order or postal order must accompany these forms*, unless a deposit has been made, one penny being allowed for each tube of blood sent. Postage is to be paid by the owner on samples sent from the farm to the laboratory.

IF ANY POSITIVE REACTORS ARE FOUND, THESE BIRDS MUST BE STRICTLY ISOLATED FROM THE REST OF THE FOWLS AND THE OWNER MUST COMMUNICATE AT ONCE WITH HIS LOCAL VETERINARY OFFICER IN ORDER TO MAKE ARRANGEMENTS WITH HIM FOR THEIR IMMEDIATE DESTRUCTION.

Please write clearly.

Box Serial No. ....

Bleeder ....

Bleeder's Address ....

Owner ....

Farm ....

Post Office ....

District ....

Date of bleeding..............................Date of despatch..............................

No. of samples in this box..

FOR OFFICE USE ONLY.

Date of Receipt..............................Ref. No..............................

(i.e. No. on Main Chart).

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

X—positive. O—unsuitable. N.—no sample.

Money received...

Remarks...

....

Signed...

Date of Test...

Date ....

....
Officer in Charge, Allerton Laboratory.

Form sent out in triplicate, one for owner, one for Government Veterinary Officer of farmers district and one kept for reference purposes.

BACILLARY WHITE DIARRHOEA TEST.

BASILLÊRE WIT DIARREE TOETS.

This form is to be filled in, together with the other three forms, and be returned with them and the samples to Allerton. This form will *not* be returned to the bleeder or owner.

Fill in the box number. Fill in the space reserved for each tube, the number of the sealed or seamless wing or legband. This form, duly filled in, will enable the Department to control properly the destruction of all reactors.

Hierdie vorm, saam met die drie ander vorms, moet ingevul, en saam met die monsters na Allerton teruggestuur word. Hierdie vorm sal *nie* aan die bloeier of eienaar teruggestuur word nie.

Vul die kas nommer in. In die spasie vir elke buisie moet die nommer van die verseëlde of naatlose vlerk- of beenband ingevul word. Hierdie vorm, behoorlik ingevul, sal die Departement in staat stel om die vernietiging van reageerders behoorlik te kontroleer.

Box No... Kas No. ....

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	67	77	78	79	80
81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100

Form sent out for recording leg or wingband numbers of fowls bled. This accompanies blood samples.

UNION OF SOUTH AFRICA.

DEPARTMENT OF AGRICULTURE AND FORESTRY.
DIVISION OF VETERINARY SERVICES.

BACILLARY WHITE DIARRHOEA TEST.

I hereby certify that during the last two tests no reactors to the above test have been found in the fowls and turkeys belonging to

Name : ....

Farm and Number : ....

Post Office : ....

District : ....

Signed :

DIRECTOR OF VETERINARY SERVICES.

Date..............................

UNIE VAN SUID-AFRIKA.

DEPARTEMENT VAN LANDBOU EN BOSBOU.
AFDELING VEEARTSENYDIENS.

BASILÊRE WIT DIARREE TOETS.

Ek verklaar hiermee dat vir die laaste twee toetse geen reageerders tot bostaande toets gevind is nie onder die hoenders en kalkoene wat behoort aan :—

Naam : ....

Plaas en Nommer : ....

Poskantoor : ....

Distrik : ....

Geteken :

DIREKTEUR VAN VEEARTSENYDIENS.

Datum..............................

BACILLARY WHITE DIARRHOEA AND FOWL TYPHOID FREE CERTIFICATE.

INSTRUCTIONS FOR USE OF INCUBATOR BOX.

To be used if atmospheric temperature is below 80° F. in the shade.

The serum should never be exposed to a temperature exceeding 100° F. unless the shade temperature exceeds this.

Under the shaded area on top a plate of asbestos is secured to the wood. This stops the wood from charring when a 100 to 150 egg incubator lamp is put into the box through the middle door. The flame is regulated so that a thermometer in the box registers about 90° F. (33 to 35° C.). The boxes of tubes are put in at both ends. The box is, of course, opened every time a tube is taken out to be filled, and when it is returned. This box will take 4 boxes each of 100 tubes.

As soon as the serum has come off well, the box of tubes can be taken out and packed for shipment to the laboratory.

In cold weather let the tubes and boracic be warmed up in the incubator before use to about 90° F.

This box should be kept out of a draught, at the bleeder's elbow.

The middle door is opened only to attend to the lamp.

Two or three ventilation holes (½ inch diameter) are bored through the wood at each end, so that the temperature in the box can be kept constant if all the doors are closed.

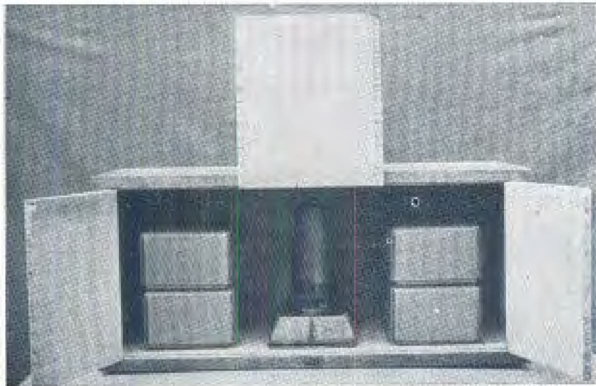
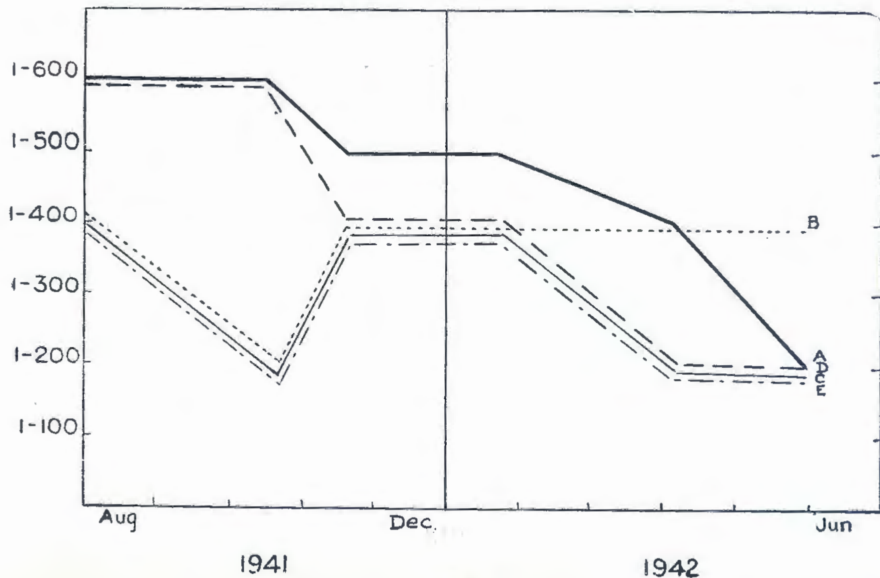
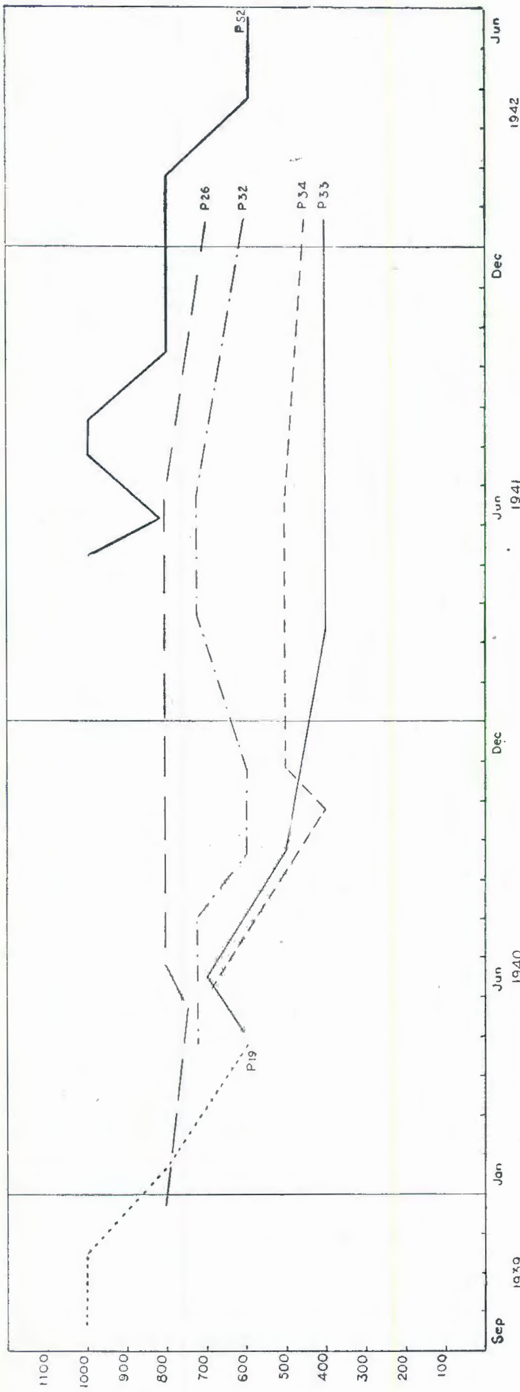


Fig. 7.—Incubator box.

GRAPH 1.—*Methods of antigen preparation—Pullorum titrations. P. 52.*

GRAPH 2.—*Methods of antigen preparation. Gallinarum titrations. G. 70.*





GRAPH 3.—*Keeping qualities of antigens and effect on titres—S. pullorum.*

GRAPH 4.—*Keeping qualities of antigens and effect on titres—S. gallinarum.*

GRAPH 5.—*Number of tests annually since* 1939.