A STUDY OF THE BIOTYPING AND BACTERIOPHAGE TYPING OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM CASES OF STAPHYLOCOCCOSIS IN POULTRY.

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SUMMARY.

135 isolates from cases of staphylococcosis in poultry were obtained from the Veterinary Investigation Centre, Lasswade, Midlothian. These isolates were purified, and divided into 4 biotypes on the basis of:

1) coagulase activity,
2) production of haemolysis on sheep blood agar,
3) pigment production on milk agar,
4) Voges Proskauer reaction,
5) fermentation of galactose, lactose and mannitol.

Materials and methods for these tests are described.

The 119 purified strains were classified into types 4, 5, 6 and 7. There was a distinct difference between the biotypes of the Lasswade isolates and those of strains typed previously by other workers in Japan.

When these types were related to the outbreaks of staphylococcosis on the farms of origin of the strains, there was no correlation between a particular biotype and a specific outbreak, or between a particular biotype and a specific farm.

8 bacteriophages were detected in the 119 staphylococcal strains. Materials and methods for their isolation, propagation, sterilisation and titration are described.

The 119 strains were phage typed, using the 8 isolated phages. 25 strains (21%) were typable at 100 x R.T.D., and these were divided into 5 phage groups. Due to the small number of strains typable, it was difficult to draw definite conclusions, but there appeared to be a relationship between the phage group of a strain and its farm of origin. The study indicated that phages derived from avian staphylococcal isolates could be of value in the typing of staphylococci from poultry.

INTRODUCTION.

Avian staphylococcosis has been reported from all parts of the world in which poultry are kept, and several clinical manifestations are described. The disease has been shown in some cases to be a secondary invasion of staphylococci following trauma or disease. However, many outbreaks have been described where the organism appears to be a primary pathogen. Recent work has therefore concentrated on the study of characters of staphylococci to ascertain whether or not outbreaks of the disease are biologically distinct.

Sato et al. (1972) classified strains of staphylococci isolated from poultry into 4 biotypes, on the basis of 6 characters. The object of the first part of this study was to investigate the biological characters of 135 samples isolated at Lasswade from cases of staphylococcosis in poultry, and attempt to classify these into biotypes using the criteria of Sato. A correlation was made between these biotypes and the farms of origin of the isolates.

In studies of the epidemiology of staphylococcal infections in man, considerable success in tracing the source of infection has been achieved by phage typing the strains isolated. Studies of this nature in poultry have been hampered by the failure to type sufficient avian strains using the human phage set. The object of the second part of this study was to attempt to isolate phages from the 135 Lasswade poultry strains, to type these strains using the phages so isolated, and to assess the results.

REVIEW OF THE LITERATURE.

GENERAL.

Prahl was the first to report widespread lameness in young geese in 1870 (Van Heelsbergen, 1929). Lucet (1892) described the condition as an acute, infectious osteoarthritis of young geese, and demonstrated *Staphylococcus aureus* as the causative agent.

Jungherr & Plastridge (1941) described an outbreak of arthritis in 5 months old chickens, characterised by unilateral or bilateral swelling of the tarsometatarsal joints. The arthritis was often accompanied by a serous synovitis. Devriese, Devos, Beumer & Maes (1972) described a similar condition in poultry in Belgium, noting inflammation of the tarsal joints, particularly the tendon sheaths in the tarsal region. Hunter (1975) observed inflammation of the tendon sheaths rather than the joints in an outbreak of staphylococcosis in poultry. Hinshaw & McNeil (1952) stated that this localisation in the joints and tendon sheaths was preceded by a septicaemia.

Vesicular dermatitis is reported by Hoffman (1939) as a syndrome of staphylococcosis. Williams & Daines (1942), Harry (1957) and Sato, Miura, Miyamae & Nakagawa (1961) described yolk sac infections in chick embryos and young chicks caused by staphylococci. Wing oedema and gangrene caused by staphylococci are described by Kawashima & Nakamura (1938), Mondini & Quaglio (1956) and Sato, Miura & Miyamae (1958). Carnaghan (1966) described spinal cord compression in fowls caused by staphylococci.

BIOLOGICAL PROPERTIES OF PATHOGENIC STAPHYLOCOCCI.

The biological properties of the staphylococci have been extensively studied. Much work has been done on their classification, and on the differentiation of pathogenic from non-pathogenic strains.

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Coagulase.

Staphylococci were discovered by Sir Alexander Ogston in 1881, and Rosenbach (1884) classified them by the colour of their colony into Staphylococcus aureus and Staphylococcus albus. This became the means of differentiation of pathogenic from non-pathogenic strains. The ability of pathogenic staphylococci to coagulate plasma in vitro was first described by Loeb (1903), but it was not until 20 years later that its use in differentiating the pathogenic and non-pathogenic strains was acknowledged by numerous authors. Smith (1947) stated that the production of coagulase is the only absolute criterion of pathogenicity among strains of staphylococci. Pathogenic strains isolated from poultry have been shown by numerous authors to coagulate rabbit plasma, (e.g. Jungherr & Plastridge, 1941; Harry, 1967; Kuramasu et al., 1967; Devriese et al, 1972), while human, bovine, ovine, equine, porcine, canine and avian plasmas are variable in their ability to be coagulated by these strains (Genigeorgis & Sadler, 1966; Harry, 1967; Kuramasu et al., 1967).

Haemolysis.

Cowan (1938) stated that there was an absolute correlation between haemolysin and coagulase production. However, Smith (1947) noted that while pathogenic staphylococci isolated from animals produced haemolysis on sheep blood agar, those isolated from poultry often failed to do so. He also noted that the production of a clear haemolysis on sheep blood agar was not restricted to pathogenic strains.

The presence or absence of haemolysis depends also on the species of the red blood cells incorporated in the agar. Nakagawa (1958) stated that sheep and cow red cells are most suitable for the detection of α and β haemolysin, while for the detection of δ-lysin, human or horse blood should be used.

Devriese et al., (1972), found that 75 out of 76 strains of pathogenic staphylococci isolated from poultry produced α haemolysin, and 89% of these strains produced both α and δ haemolysin. Sato et al., (1972) reported that 481 out of 491 coagulase positive strains that produced haemolysins produced an α haemolysin, while 65 out of 1,021 coagulase positive strains examined produced no haemolysin at all.

Pigment.

The staphylococci were originally classified by the colour of their colonies into Staph. aureus and Staph. albus (Rosenbach, 1884). However, although numerous authors record that the majority of pathogenic strains are pigmented, it became clear that this was not a sound method of distinction between pathogenic and non-pathogenic strains, as both can be pigmented (Smith, 1947), and pigment is subject to relatively frequent bacterial variation (Elek, 1959). Smith (1947), noted that 71.8% of pathogenic strains of staphylococcus were golden, while 26.4% were white, and 1.8% yellow. Fraser (1964) stated that pathogenic dog strains seldom formed aureus pigment. Sato et al., (1972), recorded that although the majority of isolates of coagulase positive staphylococci from poultry produced yellow pigment, 6.4% gave white colonies.

Voges - Proskauer reaction.

Cowan & Steel (1965) stated that Staph. aureus is positive to the Voges Proskauer (V-P) reaction. However, Cowan (1938) found that out of 13 coagulase positive staphylococci of animal origin, 9 were V-P positive and 4 were V-P negative. Sato et al., (1972), reported that 770 out of 1,021 coagulase positive staphylococci isolated from poultry were V-P negative.

Fermentation of carbohydrates.

The carbohydrates which are most consistently fermented by

staphylococci with the production of acid include glucose, lactose, maltose, mannitol, glycerol, sucrose, erythritol, fructose and mannose (Elek, 1959). Elek stated that of all these reactions, the fermentation of mannitol was claimed to show the best correlation with pathogenicity. In his classification of the staphylococci, Baird-Parker (1963) stated that coagulase positive staphylococci usually ferment mannitol. Numerous authors report that their coagulase positive isolates all ferment mannitol (e.g. Jungherr & Plastridge, 1941; Sato _et al_., 1961; Genigeorgis & Sadler, 1966; Devriese _et al_., 1972). Smith (1947), reported that 105 of 110 pathogenic staphylococci fermented mannitol, and Sato _et al_., (1972), found that of 1,021 coagulase positive strains, 65 failed to ferment mannitol.

Lactose and galactose are more variable in their fermentation reactions with pathogenic staphylococci. Smith (1947) reported that all pathogenic strains examined fermented both these sugars. Jungherr & Plastridge (1941), and Sato _et al_., (1961), found that all coagulase positive strains ferment lactose, and Kuramasu _et al_., (1967) reported 47 of 67 coagulase positive strains did not ferment galactose, and 10 of 67 coagulase positive strains did not ferment lactose. Sato _et al_., (1972), found that 699 out of 1,021 coagulase positive strains fermented neither lactose or galactose.

BIOTYPING.

Staphylococcosis has been shown in some instances to be the result of invasion of staphylococci following trauma or fowl pox (Jungherr & Plastridge, 1941). However, many outbreaks occur without apparent trauma involved (Hunter, 1974). Sato _et al_., (1958), said that outbreaks of the disease seemed to be promoted by various factors such as poor hygienic conditions, coccidiosis, or nutritional conditions.

This query as to the epidemiology of certain outbreaks led to attempts to determine if a particular strain of staphylococcus was associated with a particular outbreak.

Kuramasu et al., (1967) reported that it was impossible to find any correlation between the results of carbohydrate fermentation, and the origin of the strains. Sato et al., (1972), attempted to classify 1,021 strains obtained from 27 farms into biotypes on the basis of six characters. These characters were pigmentation of colony, haemolysis on sheep blood agar, Voges-Proskauer reaction, and the fermentation of lactose, galactose and mannitol. He succeeded in classifying the strains into four biotypes, and reported that generally speaking, a uniformity of biological types was observed among strains derived from a single flock.

PHAGE TYPING.

The action of bacteriophage on staphylococcal cultures was first described by Twort in 1915 (Elek, 1959). Epstein and Fejgin noted in 1926 that there were differences between staphylococci obtained from lesions and other sources as regards their susceptibility to phage action (Elek, 1959). The systematic use of phages for epidemiological investigation of staphylococcal infection was first employed by Fisk (1942, a, b). Wilson and Atkinson (1945) extended this work, using 18 phages to examine various outbreaks of food poisoning and pemphigus neonatorum, where they were able to trace the source of infection by detecting identical phage types in isolates from carriers and from affected patients.

In the veterinary field, the use of phage typing was first recorded by Smith (1948). He examined 1,016 strains of staphylococcus from bovine milk samples, and reported that 93.3% of the strains were phage-typable, and 6.7% were not. It was unusual to find one phage type predominant in the strains isolated from the milk samples of any one herd. Nakagawa (1960), reported that of 375 strains of staphylococci from bovine milk samples, 171 (45.6%) were typed by one or more of the twenty human phages used. He

went on to suggest that several of the human phages were unsuitable for the typing of bovine milk. When 442 milk strains were tested using bovine phages, 319 strains (72.2%) were typable (Nakagawa, 1960). Davidson (1961) produced a set of phages specific for bovine strains which he suggested would be more useful than the human phage set. Markham & Markham (1966), reported that the human phage set typed 78% of human strains of staphylococci, 53% of bovine strains, 25% of hedgehog strains and 8% of sheep strains. However, bovine phages typed 92% of bovine strains. They suggested that staphylococci tend to be species specific, and that for most species it will be necessary to develop a separate phage set, although some phages will have a place in more than one set. Similarly, Watson (1964), examining strains of staphylococci from sheep, suggested that it would be desirable to have a phage typing system specifically designed for sheep strains. Oeding et al., (1970), reported that no staphylococcal strains of canine origin were typable with human or bovine phages, confirming that canine strains were biochemically separate from human and bovine strains.

With pathogenic staphylococci isolated from poultry, similarly poor results have been obtained using the human phage set. Sato et al., (1961), found that 6 of 71 coagulase positive staphylococci isolated from poultry were typable using the international set of human phages, and 2 of the 71 strains were slightly lysed using bovine phages. Kuramasu et al., (1967), reported that none of the coagulase positive strains isolated from cases of staphylococcosis were typable using N.C.T.C. phages at 100 x R.T.D.

Genigeorgis & Sadler (1966) reported 22 out of 25 coagulase positive strains isolated from livers of commercially slaughtered poultry were typable using human phages. Harry (1967) found 23% of fowl strains typable at routine test dilution (R.T.D.) and 54% typable at 1,000 x R.T.D. He found no relationship existed between phage patterns of strains originating from the same geographical area.

The methods for the phage typing of staphylococci have been extensively reviewed (Blair & Williams, 1961; Wentworth, 1963), as have the interpretation of the results, (Anderson & Williams, 1956; Blair & Carr, 1960). Blair & Williams (1961) in their method of recording phage typing, did not report any reactions showing less than 50 plaques, while Ruys & Borst (1959) suggested a more complicated, but more informative method of reporting, where all visible reactions are noted in order of conspicuity. Ruys and Borst feel that the tail of weaker reactions is extremely important for true differentiation of strains, and is of epidemiological significance.

TABLE I.

FARMS OF ORIGIN OF STAPHYLOCOCCAL ISOLATES, INCLUDING SAMPLE NUMBERS IN EACH OUTBREAK OF DISEASE.

FARM	SAMPLE NOS., AND OUTBREAKS.
A	1,2,3,6,8,9,11,13/ 49,50,51/ 62,67,77,83/ 89/ 111/ 123/
B	19,30,38,55/ 36/ 103/ 107/ 108/ 109/
C	21,29/ 70/
D	17,18/ 27/ 37/ 45,46/ 56/ 58/ 66/ 72,78/ 92/ 100/ 106/ 112,118,124,125/
E	26/ 39/ 53,54,60,61/ 80,84/ 105/ 130/
F	10/
H	4/ 5,7/ 14,16/ 15/ 20/ 22,23,25,28/ 34,35/ 42/ 64,82/ 73/ 88/ 95/ 96/ 99/ 104/ 110,116,117/ 122,127/ 132,134,135/
J	40/ 41/ 98/ 101,102/
K	44/
L	48,52,57/ 65/
M	59/ 63,68,69,71,74,75,79,81,85,86/ 93/ 128,129,133/
N	113,114,119,120,126/
O	121/

Sample no. 47 isolated from a turkey.
Sample no. 90 isolated from an owl.

MATERIALS AND METHODS.

1. SAMPLES.

135 specimens isolated from cases of staphylococcosis in poultry were obtained from the Ministry of Agriculture Veterinary Investigation Centre at Lasswade, Midlothian.

These were supplied as cultures on nutrient agar slopes, and were stored in the refridgerator at 4° C. They had been isolated from lesions in poultry sent in for post mortem examination between December 1973 and January 1975. (see Table I). Isolations were made from the joints, tendon sheaths, and livers of affected birds.

Purification.

Each isolate was streaked out on to a 2 % sheep blood agar plate. After incubation for 24 hours at 37° C., single staphylococcal colonies were removed on a sterile nickel loop, and sown on to nutrient agar slopes.

The identity of the colonies was confirmed by making a smear of each colony on a microscope slide, fixing briefly in a bunsen flame, staining by Gram's method, and examining the slides under a microscope using the x 100 objective. Gram-positive cocci, often in clusters, were seen in pure culture.

2. EXAMINATION OF BIOLOGICAL CHARACTERISTICS.

a) COAGULASE ACTIVITY.

Rabbit plasma (Wellcome) was used. The dried reagent was reconstituted with 0.5 ml. sterile water, and was diluted 1:10 with 0.9 % saline. 0.5 ml. was added to a 3" x 1/2" test tube, in a water bath at 37° C.

5 drops of an 18 hour broth culture of the test sample were added, and the tubes incubated at 37° C. and observed for coagulation at 1, 3 and 6 hours.

b) HAEMOLYSIS ON SHEEP BLOOD AGAR.

Each purified isolate was streaked out on to a 2% sheep blood agar plate, and incubated at 37° C. for 24 hours. After incubation, the presence or absence of any haemolysis of the sheep blood agar was noted.

c) PIGMENT PRODUCTION.

Each purified isolate was streaked out on to a 10% skimmed milk agar plate, and incubated at 37° C. for 24 hours. The plates were then left on the bench for 3 days, before being examined for the presence or absence of pigment in the colony.

d) VOGES PROSKAUER REACTION.

The method of Barrit (1936) was used. 10 ml. of MRVP medium (Oxoid) were inoculated with 2 loopfuls of the sample under test, and then incubated at 37° C. for 48 hours. After incubation, 1 ml. of the solution was transferred to a test tube, and to it, 0.6 ml. of napthol solution, and 0.2 ml. 40% potassium hydroxide aqueous solution, were added. The tube was shaken, and examined after 15 minutes. A positive reaction was indicated by a strong red colour. MRVP medium inoculated with <u>Enterobacter aerogenes</u> was used as a positive control.

e) FERMENTATION OF GALACTOSE, LACTOSE AND MANNITOL.

Solutions of galactose, lactose and mannitol were prepared in Andrades peptone water (Oxoid) to give a final concentration of 1% of the sugar. These were sterilised by filtration, and dispensed into bijou bottles.

Using sterile nickel loops, each sample was inoculated into the three sugars in turn, and these were incubated at 37° C. for 24 hours. Fermentation of the sugars was indicated by the solution turning pink.

TABLE II.

CROSS CULTURE TECHNIQUE (Fisk, 1942).

SAMPLES USED.

1st Screening	5,10,20,25,30,35,40,50, 60,70,77,90,100,110,120,130.
2nd Screening	1,17,19,21,28,36,39,44,48, 49,67,69,98,109,119,123,124.
3rd Screening	7,9,14,22,26,27,45,56,64, 68,72,81,84,95,99,133,135.
4th Screening	1,5,6,10,28,36,40, 55,63,70,77,90,110.

3. DETECTION OF BACTERIOPHAGE.

To detect any bacteriophage present in the specimens isolated, the cross culture method (Fisk, 1942) was used, with modifications.

16 samples were chosen at random. (see Table II, 1st screening). Each was inoculated into nutrient broth (No. 2, Oxoid), and incubated at 37° C. for 18 hours.

8 to 10 drops of a broth culture were pipetted on to a nutrient agar plate (Oxoid), spread over the whole of the plate, and the excess discarded.

The plate was then dried in the incubator for 1 hour, with the lid of the plate slightly open. When dry, the other 15 broth cultures were spotted on to the plate, using sterile pasteur pipettes.

This process was repeated using each of the 16 broth cultures in turn as the basal strain. The plates were then incubated overnight at 30° C.

Screening was continued using further sets of samples, chosen at random. (see Table II, 2nd - 4th screenings).

In all cases, the presence of phage was revealed the morning after incubation of the plates by the presence of either discrete plaques, or a narrow zone of inhibition of growth around the spot.

4. PREPARATION OF CRUDE PHAGES.

The method of Williams Smith (1948), was used.

Having identified the presence of phage by the cross culture method, the pair of samples involved were plated together as follows.

One sample was used as the basal strain, and dried on to a nutrient agar plate as described. The other strain was then spotted on.

After incubation at 30° C. overnight, the plaques were scraped off the plate, and suspended in 5 ml. nutrient broth. This was then centrifuged at 3,000 r.p.m. for 15 minutes.

The supernatent was withdrawn, and labelled crude phage.

Nutrient agar plates were then sown with the two parent strains, and dried. The crude phage was then spotted on to both parent strains, and incubated overnight at 30° C. This was to determine which was the lysogenic, and which the susceptible strain. Plaques were found on the susceptible sample, but not on the lysogenic.

5. PURIFICATION OF PHAGES.

The method of Williams Smith (1948) was used.

A single plaque was removed from the susceptible strain, after incubation with the crude phage, as described above. This was spread on the surface of a nutrient agar plate. The plate was incubated at 30° C. overnight, and the growth present the next day was scraped off with a sterile nickel loop, and suspended in 5 ml. nutrient broth. This was centrifuged at 3,000 r.p.m. for 15 minutes. The supernatent was withdrawn, and stored in a refridgerator at 4° C. before propagation.

6. PROPAGATION OF THE PHAGES.

Propagation of the phages was done using the soft agar technique (after Swanstrom and Adams, 1951).

15 cm. petri dishes, containing nutrient agar, were used.

The soft agar was prepared as follows.

Nutrient broth was made up. To this was added 0.5 gm/100 ml. granular agar (Ionagar No. 2, Oxoid), and 400 μg/ml. of calcium chloride. The mixture was cooled to 45° C. in a water bath.

To this were added the cells from a 6 hour nutrient broth culture of the propagating (susceptible) strain. These cells were obtained by centrifugation of the broth culture at 3,000 r.p.m. for 10 minutes.

2 ml. of the purified crude phage, to which the propagating strain was susceptible, was added, and the mixture shaken gently.

10 ml. of this soft agar/culture/phage mixture were then transferred to the surface of a nutrient agar plate, using a sterile pipette.

The plates so prepared were incubated for 18 hours at 30° C.

After incubation, 20 ml. of nutrient broth were added to each plate. The soft agar layer was stirred up with a sterile bent glass rod, and the soft agar pieces and broth were poured into sterile universal containers. The mixture was shaken vigourously to break up the agar lumps, and then centrifuged at 5,000 r.p.m. for 20 minutes. The supernatent, purified propagated phage, was withdrawn.

7. STERILISATION OF LYSATES.

The supernatent lysates so produced were then sterilised by filtration. This was done by drawing up the supernatent into a 20 ml. syringe, and attaching to the syringe a disposable millipore filter unit, (Millex, Millipore Corporation, Bedford, Massachusetts), with pore size of 0.22 μ. The lysate was passed gently through the filter, into a sterile universal container, and then stored in the refridgerator at 4° C.

8. TITRATION OF PHAGE FILTRATES.

All the phage filtrates so produced were then titrated as follows.

Each filtrate was diluted out in 10 fold dilutions in sterile nutrient broth from concentrate to 10^{-6}.

Nutrient broth cultures of the propagating strains were prepared, and incubated overnight at 37° C. These were then flooded on to nutrient agar plates, the excess removed, and the plates were dried in the incubator for 1 hour, with the plate lids slightly open.

The phage filtrate dilutions were then spotted on to their respective propagating strains on the nutrient agar plates, using a pasteur pipette to dispense 0.02 ml. of the filtrate.

The plates were then incubated at 30° C. overnight, and the routine test dilution (R.T.D.) of the phage was read as that dilution which just failed to give confluent lysis of the propagating strain.

9. PHAGE TYPING.

The method of Blair and Williams (1961) was used.

Nutrient broth cultures of all the samples were prepared, and incubated overnight at 37° C.

Base lawns of each sample were then prepared and dried on nutrient agar, as described above.

The phage filtrates were then spotted on to the dried lawns, each at R.T.D. and 100 x R.T.D., using a new sterile 1 ml. syringe and 26 guage needle for each phage dilution.

The plates were then incubated at 30° C. overnight, and were read and reported the following morning.

Lysis by any of the phage filtrates was reported as follows.

Confluent lysis to 50 plaques .... ++
20 - 50 plaques .... +
Less than 20 plaques .... ±

Typing was done by 2 methods, both at R.T.D. and 100 x R.T.D.

1. Method of the Subcommittee on Phage Typing of Staphylococcus of the Nomenclature Committee of the International Association of Microbiological Societies (Blair & Williams, 1961).

The phage type of the strain was recorded by this method by listing the numbers of those phages which gave a ++ reaction, and separating these numbers by oblique lines, e.g. 56/28/10.

2. Method of Ruys and Borst (1959). This method involved the reporting of all visible reactions. The phages giving complete lysis were recorded first, followed by a slant line; then those which gave at least a ++ reaction, followed by a colon; next, reactions which were still easily read, followed by a slant line; and finally, the weak reactions, e.g. 56,28,10/ - : - / 81.

10. ELECTRON MICROSCOPY.

A solution of purified propagated phage was centrifuged at 35,000 g for 30 minutes. The supernatent was withdrawn, and a small amount of fluid was left in the tube. The fluid was removed to a shallow container.

Carbon coated copper grids were floated on the suspensions for 30 seconds, and excess fluid was removed by touching a filter paper to the grid edge. The grids were then floated on a solution of phosphotungstic acid (P.T.A.) at $_pH$ 7.7. Excess P.T.A. was removed with filter paper.

The grids were then examined under an electron microscope.

RESULTS.

1. PURIFICATION.

Of the 135 samples, 119 were strains of staphylococcus sp. in pure culture. 16 strains were either gram -ve rods, or were heavily contaminated with a gram -ve organism, and were discarded.

These strains were the following sample numbers:

23	34	37	41	47	65	89	91
92	101	102	104	106	111	115	128.

2. BIOLOGICAL TYPING.

a) COAGULASE ACTIVITY.

All the 119 strains coagulated rabbit plasma. Nearly all the strains produced firm clots, not easily disrupted by shaking, within three hours of incubation.

b) HAEMOLYSIS ON SHEEP BLOOD AGAR.

Of the 119 strains, 49 produced a clear zone of haemolysis when incubated on sheep blood agar (41.2%). 70 (58.8%) gave no haemolysis.

c) PIGMENT PRODUCTION.

92 of the 119 strains (77.3%) produced yellow pigment when incubated on milk agar. 27 of the strains (22.7%) produced no pigment, and were white colonies on milk agar. Although the majority of strains were easily read as yellow or white, some difficulty was experienced over certain pale cream colonies. These were classified as yellow or white after a standard set up by the author.

d) VOGES PROSKAUER REACTION.

All 119 strains (100%) were negative to the Voges Proskauer test.

e) FERMENTATION OF GALACTOSE, LACTOSE AND MANNITOL.

Galactose: 109 of the 119 samples (91.6%) fermented galactose, and 10 (8.4%) failed to do so.
Lactose: 113 of the 119 samples (95%) fermented lactose, 3 did so very weakly (2.5%) and 3 failed to do so altogether (2.5%).
Mannitol: 116 of the 119 samples (97.5%) fermented mannitol, and 3 (2.5%) failed to do so.

The reactions of each strain are shown in Table III, and this is summarised in Table IV.

TABLE III.

BIOLOGICAL REACTIONS OF STAPHYLOCOCCI ISOLATED FROM POULTRY AT LASSWADE.

Sample	Haemolysis	Pigment	V-P[3]	L[4]	M[5]	G[6]	Coagulase
1.	-	Y[1]	-	+	+	+	+
2.	+	Y	-	+	+	+	+
3.	+	Y	-	+	+	+	+
4.	-	Y	-	+	+	+	+
5.	+	W[2]	-	+	+	+	+
6.	+	Y	-	+	+	+	+
7.	-	Y	-	+	+	+	+
8.	-	W	-	+	+	+	+
9.	-	Y	-	+	+	+	+
10.	+	Y	-	+	+	+	+
11.	+	W	-	+	+	+	+
12.	-	Y	-	+	+	+	+
13.	+	Y	-	+	+	+	+
14.	-	W	-	+	+	+	+
15.	+	W	-	+	+	+	+
16.	-	W	-	+	+	+	+
17.	-	Y	-	+	+	+	+
18.	-	Y	-	+	+	+	+
19.	-	Y	-	+	+	+	+
20.	-	W	-	-	+	-	+
21.	-	Y	-	+	+	+	+
22.	-	Y	-	+	+	+	+
23.							
24.	+	Y	-	+	+	+	+

[1]Y = Yellow [2]W = White [3]V-P = Voges Proskauer [4]L = Lactose
[5]M = Mannitol [6]G = Galactose

TABLE III (contd.)

Sample	Haemolysis	Pigment	V-P	L	M	G	Coagulase
25.	-	Y	-	-	-	-	+
26.	+	Y	-	+	+	+	+
27.	-	W	-	+	+	+	+
28.	-	Y	-	+	+	+	+
29.	-	Y	-	+	+	+	+
30.	-	Y	-	+	+	+	+
31.	-	Y	-	+	+	+	+
32.	-	Y	-	+	+	+	+
33.	-	Y	-	+	+	+	+
34.							
35.	-	Y	-	+	+	+	+
36.	+	Y	-	+	+	+	+
37.							
38.	-	Y	-	+	+	+	+
39.	-	Y	-	+	+	+	+
40.	-	Y	-	+	+	+	+
41.							
42.	-	Y	-	+	+	+	+
43.	-	Y	-	+	+	+	+
44.	-	Y	-	+	+	+	+
45.	+	Y	-	+	+	+	+
46.	-	Y	-	+	+	+	+
47.							
48.	-	Y	-	+	+	+	+
49.	+	Y	-	+	+	+	+
50.	+	Y	-	+	+	+	+
51.	-	Y	-	+	+	+	+
52.	-	Y	-	+	+	+	+
53.	+	Y	-	+	+	+	+

TABLE III (contd.)

Sample	Haemolysis	Pigment	V-P	L	M	G	Coagulase
54.	+	Y	–	+	+	+	+
55.	+	Y	–	+	+	+	+
56.	+	Y	–	+	+	+	+
57.	+	Y	–	+	+	+	+
58.	+	Y	–	+	+	+	+
59.	–	W	–	+	+	+	+
60.	+	Y	–	+	+	+	+
61.	+	Y	–	+	+	+	+
62.	–	W	–	+	+	+	+
63.	+	Y	–	+	+	+	+
64.	–	Y	–	+	+	+	+
65.							
66.	+	W	–	+	+	–	+
67.	+	W	–	+	+	+	+
68.	+	Y	–	+	+	+	+
69.	+	Y	–	+	+	+	+
70.	+	Y	–	+	+	+	+
71.	+	Y	–	+	+	+	+
72.	–	Y	–	+	+	+	+
73.	+	W	–	+	+	+	+
74.	+	W	–	+	+	+	+
75.	+	Y	–	+	+	+	+
76.	–	W	–	+	+	+	+
77.	–	Y	–	+	+	–	+
78.	–	Y	–	+	+	+	+
79.	+	Y	–	+	+	+	+
80.	+	Y	–	+	+	+	+
81.	+	Y	–	+	+	+	+

TABLE III (contd.)

Sample	Haemolysis	Pigment	V-P	L	M	G	Coagulase
82.	-	Y	-	+	+	+	+
83.	-	Y	-	+	+	-	+
84.	+	W	-	+	+	+	+
85.	+	Y	-	+	+	+	+
86.	+	Y	-	+	+	+	+
87.	-	Y	-	-	-	-	+
88.	+	Y	-	+	+	+	+
89.							
90.	+	Y	-	+	+	+	+
91.							
92.							
93.	-	W	-	+	+	+	+
94.	+	W	-	+	+	+	+
95.	-	Y	-	+	+	+	+
96.	-	Y	-	+	+	+	+
97.	-	Y	-	+	+	+	+
98.	-	Y	-	+	+	+	+
99.	-	Y	-	+	+	+	+
100.	-	Y	-	+	+	+	+
101.							
102.							
103.	-	W	-	+	+	+	+
104.							
105.	-	W	-	+	+	+	+
106.							
107.	-	Y	-	+	+	+	+
108.	+	Y	-	+	+	+	+
109.	-	Y	-	+	+	+	+
110.	-	Y	-	+	+	+	+
111.							

TABLE III (contd.)

Sample	Haemolysis	Pigment	V-P	L	M	G	Coagulase
111.							
112.	+	Y	−	+	+	+	+
113.	−	Y	−	+	+	+	+
114.	−	W	−	+	+	+	+
115.							
116.	−	W	−	+	+	+	+
117.	−	Y	−	±	+	−	+
118.	−	Y	−	+	+	+	+
119.	−	Y	−	±	+	−	+
120.	+	Y	−	+	+	+	+
121.	−	Y	−	+	+	+	+
122.	−	Y	−	+	+	+	+
123.	−	W	−	+	−	+	+
124.	+	Y	−	+	+	+	+
125.	−	Y	−	±	+	−	+
126.	+	W	−	+	+	+	+
127.	+	Y	−	+	+	+	+
128.							
129.	−	Y	−	+	+	+	+
130.	−	W	−	+	+	+	+
131.	+	W	−	+	+	+	+
132.	+	Y	−	+	+	+	+
133.	−	Y	−	+	+	+	+
134.	−	Y	−	+	+	+	+
135.	−	W	−	+	+	+	+

TABLE IV.

SUMMARISED RESULTS OF BIOLOGICAL TESTS ON 119 STAPHYLOCOCCAL ISOLATES OBTAINED FROM POULTRY AT LASSWADE.

Test	No. positive (% of total).	No. negative (% of total).
Coagulase	119 (100%)	0 (0%)
Haemolysis	49 (41.2%)	70 (58.8%)
Pigment	92 (77.3%)	27 (22.7%)
Voges Proskauer	0 (0%)	119 (100%)
Fermentation of:		
Galactose	109 (91.6%)	10 (8.4%)
Lactose	116+ (97.5%)	3 (2.5%)
Mannitol	116 (97.5%)	3 (2.5%)

\+ includes 3 (2.5%) very weak positives.

Table V shows the biotypes of chicken coagulase - positive staphylococci (Sato *et al*, 1972).

The author attempted to divide the 119 strains isolated at Lasswade into biotypes on the basis of the criteria of Sato *et al* (1972).

The results of this are shown in Table VI. None of the Lasswade isolates fitted in to types 1 - 3 of Sato's typing. 38 were classified as type 4, and the remainder were grouped into 3 new biotypes, 5, 6 and 7. 11 other isolates were untypable.

Biological types of chicken coagulase-positive staphylococci (Sato et al, 1972)

Biological type	Pigment	Haemolysis★	V-P reaction	Fermentation of☆		
				L	G	M
1	yellow	+	—	—	—	+
2	white	—	—	+	+	—
3	yellow	+	+	+	+	+
4	yellow	+	—	+	+	+

★ on sheep blood agar
☆ L = lactose
G = galactose
M = mannitol

TABLE V

Biological types of coagulase-positive staphylococci isolated from poultry at Lasswade

Biological type		No. of isolates	Pigment	Haemolysis☆	V-P reaction	Fermentation of★		
						L	G	M
4		38	yellow	+	—	+	+	+
5		47	yellow	—	—	+	+	+
6		10	white	+	—	+	+	+
7		13	white	—	—	+	+	+
others	a	2	yellow	—	—	—	—	—
	b	1	white	—	—	—	—	+
	c	3	yellow	—	—	+	—	+
	d	1	white	—	—	+	+	—
	e	3	yellow	—	—	∓	—	+
	f	1	white	+	—	+	—	+
Total		119						

☆ on sheep blood agar
★ L = lactose G = galactose M = mannitol

TABLE VI

The biotypes of the isolates were then related to the outbreak of disease on the farm of origin, as shown in Table VII.

TABLE VII.

BIOTYPES OF STAPHYLOCOCCUS ISOLATES RELATED TO OUTBREAKS OF STAPHYLOCOCCOSIS.

Farm	Biotypes (outbreaks divided by oblique line).
A	5,4,4,4,7,5,6,4/ 4,4,5/ 7,6,c,c/ $?^{+}$/ $?^{+}$/ d/
B	5,5,5,4/ 1/ 7/ 4/ 5/
C	5,5/ 4
D	5,5/ 7/ $?^{+}$/ 4,5/ 4/ 4/ f/ 5,5/ $?^{+}$/ 5/ $?^{+}$/ 4,5,4,e/
E	4/ 5/ 4,4,4,4/ 4/ 6/ 7/ 7/
F	4/
H	5/ 6,5/ 7,7/ 6/ b/ 5,$?^{+}$,a,5/ $?^{+}$,5/ 5/ 5,5/ 6/ 4/ 5/ 5/ 5/ $?^{+}$/ 5,7,e/ 5,4/ 4,5,7/
J	5/ $?^{+}$/ 5/ $?^{+}$,$?^{+}$/
K	5/
L	5,5,4/ $?^{+}$/
M	7/ 4,4,4,4,6,4,4,4,4,4/ 7/ $?^{+}$,5,5/
N	5,7,e,4,6/
O	5/

$^{+}$ Sample not staphylococcus.

There was no consistent correlation between a particular farm and a particular biotype, or a particular outbreak and a particular biotype. With the exception of one outbreak on farm E, (strains 53, 54,60,61), each outbreak where more than one sample was taken contained at least two different biotypes. In an outbreak on farm M, nine out of ten of the strains isolated belonged to biotype 4.

3. PHAGE ISOLATION.

The results of 4 screenings of the Lasswade staphylococcal strains, using the cross culture technique, are shown in Table VIII.

The cross culture technique, 4th screening, is illustrated in Figure I.

Using the methods described, the following phages were prepared:

90/5	64/81
110/77	56/95
40/10	28/1
70/90	1/10

(lysogenic strains underlined).

Repeated attempts to cultivate phage from strain 36 failed. Lysis was always evident in the cross culture technique in association with this strain. However, when propagation was attempted, using strains 69, 109, 124 and 135 in turn as propagating strains, the resultant "phage" repeatedly failed to give any lysis when tested against its parent propagating strain.

TABLE VIII.

CROSS CULTURE RESULTS.

1st Screening.			2nd Screening.		
Base	Spot	Activity	Base	Spot	Activity
90	5	++	1	28	++
90	70	±	1	36	++
90	110	++	17	36	++
77	110	++	19	36	++
110	5	++	21	36	++
110	77	+	28	36	++
110	90	+	39	36	++
120	40	+	44	36	++
10	40	++	48	36	++
5	90	+	49	36	++
5	110	+	67	36	++
100	40	±	69	36	++
40	10	+	98	36	++
35	40	±	109	36	++
			119	36	±
			123	36	±
			124	36	++

TABLE VIII (contd.)

CROSS CULTURE RESULTS.

3rd Screening.

Base	Spot	Activity
7	56	++
27	56	++
64	68	++
66	81	++
68	64	++
81	64	++
95	56	++

4th Screening.

Base	Spot	Activity
1	10	±
1	28	++
1	36	++
6	36	++
5	36	++
5	110	++
10	36	++
10	40	++
28	36	++
36	ALL	++
40	36	++
55	36	++
63	36	++
63	90	±
70	36	++
77	36	++
77	110	++
90	36	++
90	110	++
90	70	±
90	63	±
110	5	++
110	36	++

Fig. I

Cross culture method

a) Base lawn: Sample 1.

Lytic activity seen with samples 10, 28 and 36.

Plate read:

Base	Spot	Activity
1	10	±
1	28	++
1	36	++

b) Base lawn: Sample 90.

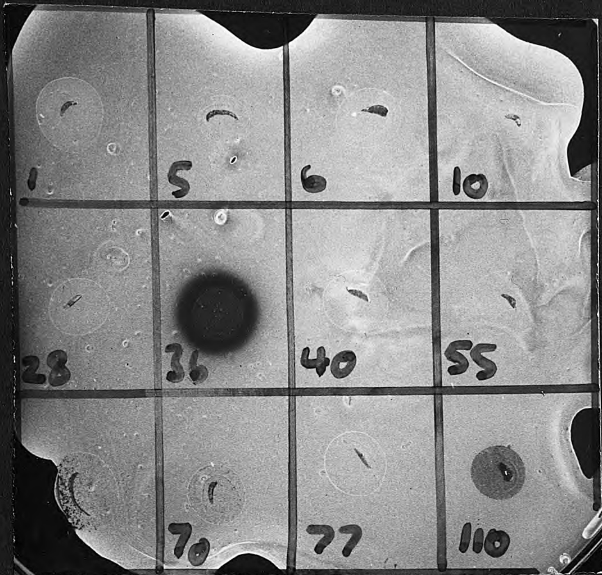
Lytic activity seen with samples 36, 63, 70 and 110.

Plate read:

Base	Spot	Activity
90	36	++
90	63	±
90	70	±
90	110	++

FIG. I.

a



b

4. PHAGE TITRATION.

The 8 phages isolated were titrated, and the routine test dilutions (R.T.D.'s) were as follows.

PHAGE	R.T.D.
5	concentrate
10	10^{-3}
28	10^{-3}
56	10^{-5}
81	10^{-5}
70	10^{-2}
40	10^{-5}
110	concentrate

The phage titrations are illustrated in Figs. II and III.

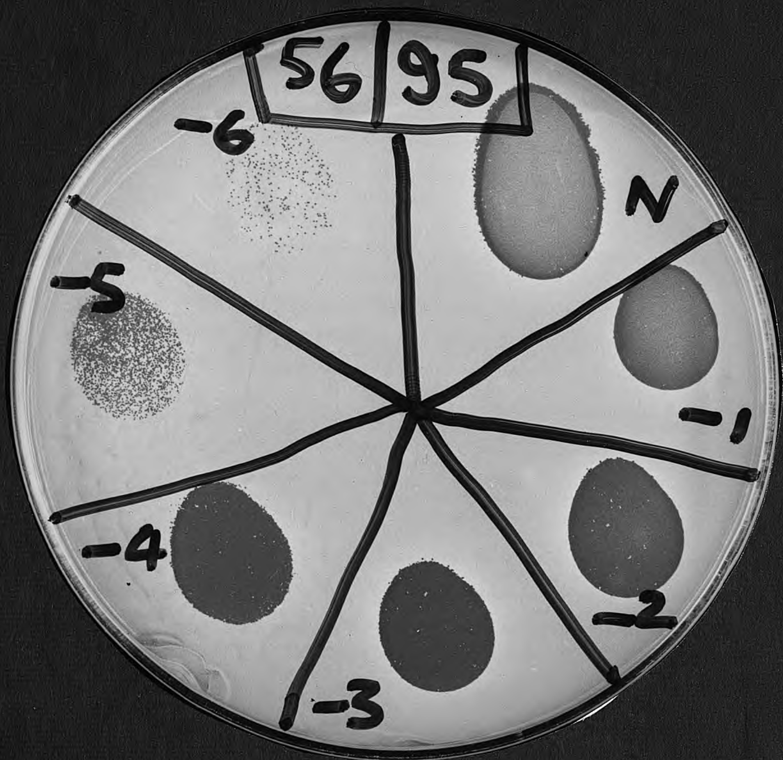
Fig. II

Phage titration.

a) Phage 56 titrated on propagating strain 95.
Dilutions of concentrate (N) to 10^{-6}.
R.T.D. read as 10^{-5}.

b) Phage 40 titrated on propagating strain 10.
Dilutions of concentrate (N) to 10^{-6}.
R.T.D. read as 10^{-5}.

FIG. II.



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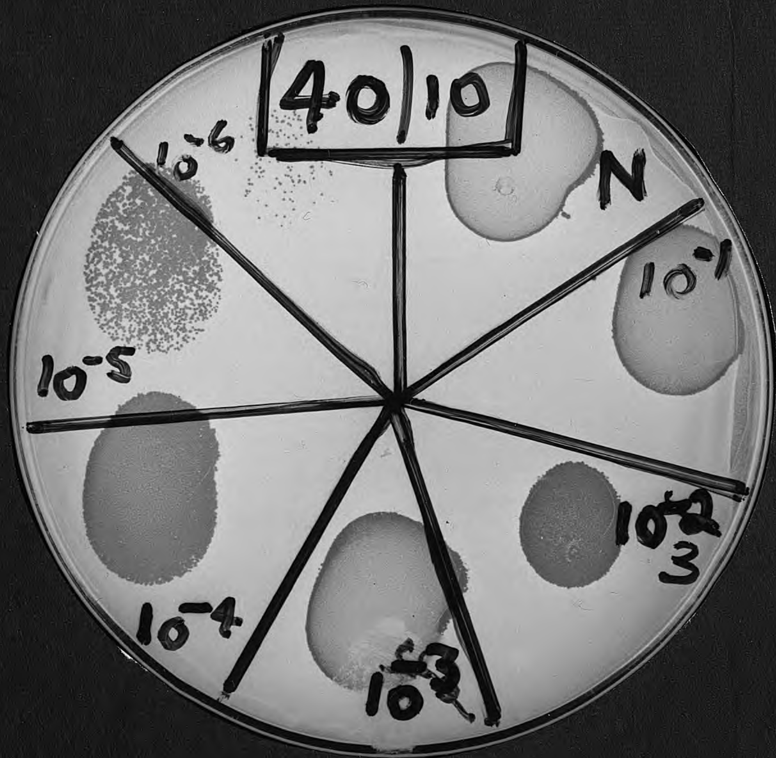


b

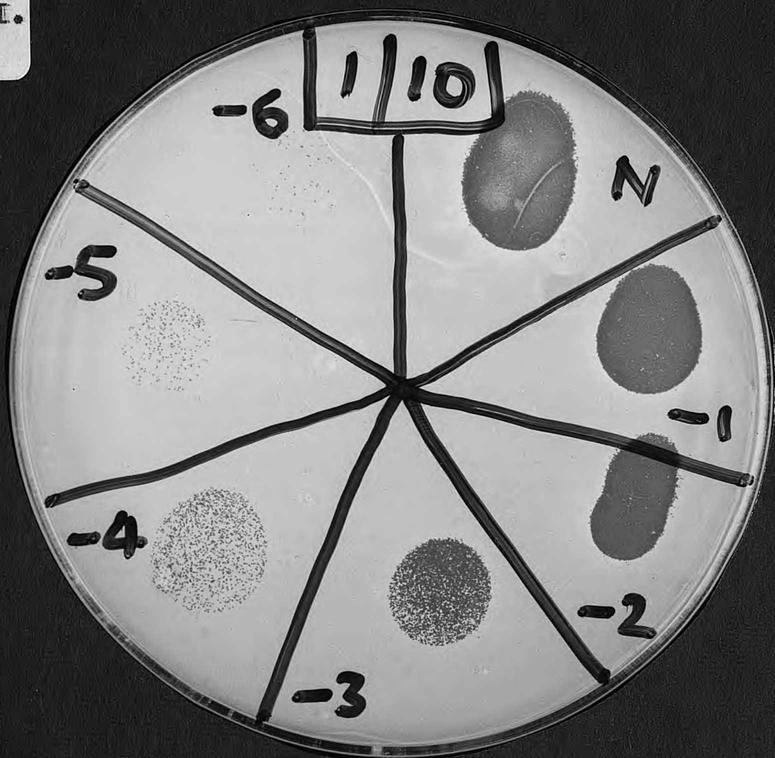
Fig. III

Phage titration (contd.)

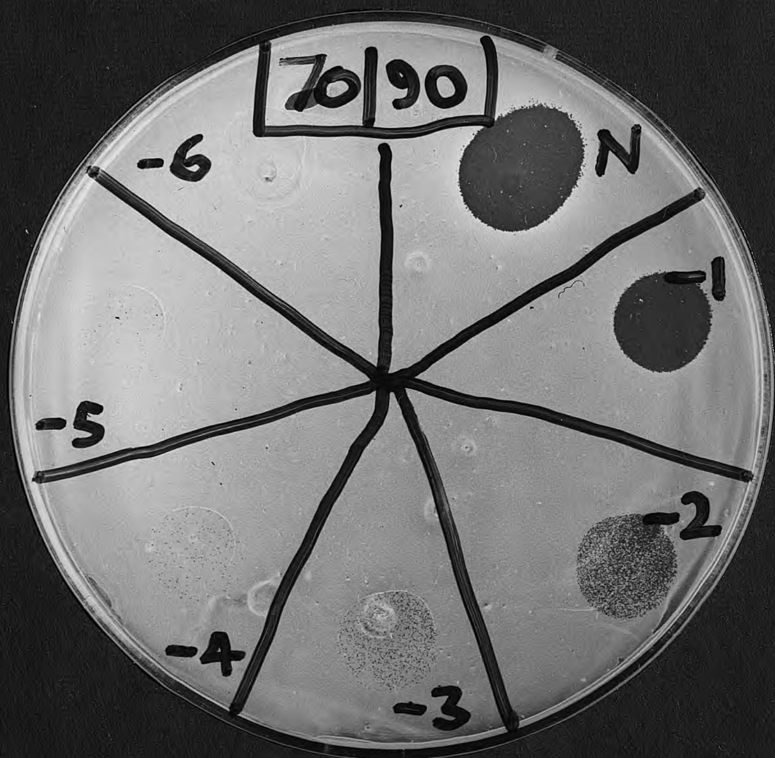
a) Phage 10 titrated on propagating strain 1.
Dilutions of concentrate (N) to 10^{-6}.
R.T.D. read as 10^{-3}.

b) Phage 70 titrated on propagating strain 90.
Dilutions of concentrate (N) to 10^{-6}.
R.T.D. read as 10^{-2}.

FIG. III.



a



b

5. PHAGE TYPING.

46 of the 119 staphylococcal strains (38.6%) were lysed by one or more of the 8 phages. Table IX shows the lytic pattern of these 46 strains, with phages at R.T.D. and 100 x R.T.D.

Table X shows the designation of phage types. Using the method of reporting of Ruys and Borst (1959), 29 strains (24.3%) were typable at R.T.D., and 46 strains (38.6%) at 100 x R.T.D. With the method of the International Association of Microbiological Societies (Blair & Williams, 1961), 0 of the 119 strains were typable at R.T.D., while 25 (21%) were typable at 100 x R.T.D.

The phage typing of strains 58, 64, 73, 77, 95, 114, 127 and 135 are illustrated in Figs. IV and V.

Phages 5 and 110 could only be propagated at R.T.D., so no samples were tested at 100 x R.T.D. with these phages.

TABLE IX.

PHAGE PATTERNS OF LASSWADE ISOLATES.

Isolate	R.T.D.								100 x R.T.D.							
no.	5	110	40	70	81	56	28	10	5	110	40	70	81	56	28	10
1								±						+	+	++
4															±	
6						±								++		
7														±	±	±
10			±			±					++			++		
21											±					
22														±		
24													+			
27														±	±	±
33	±	±			±			±					±		±	±
35		±				±		±						+	++	+
38						±		±			±			±		±
42						±										
45						+								++		
51			±	±				±			±	±				±
52	±	±		±		±						±		±		
56								±							+	+
58					+								++			
61															±	
64					+								++			
68															±	
70																±
73														±	++	+
74												±				
77		±		±								++				
79																±
83		±		±								++				

TABLE IX (contd.)

PHAGE PATTERNS OF LASSWADE ISOLATES.

Isolate no.	R.T.D. 5	110	40	70	81	56	28	10	100 x R.T.D. 5	110	40	70	81	56	28	10
88					+								++			
90				±								++				
95						±	±	±						++	++	++
100							±	±						+	++	++
105														+	++	++
108														±	+	++
110	+												±			
114			±			±					++			++		
116						±	±	±						++	++	++
120			±								++			+		
121	+												+			
122	±				±	±	±	±					±	++	++	++
127						±	±	+						++	++	++
129														±	±	±
130						±		±						++	++	++
131												+				
132					±								++			
134							±	±						++	++	++
135						±	±	±						++	++	++

TABLE X.

PHAGE TYPING OF LASSWADE ISOLATES.

Isolate no.	Method of Ruys and Borst (1959).		Method of the International Association of Micro-biological Societies.	
	R.T.D.	100 x R.T.D.	R.T.D.	100 x R.T.D.
1.	-/-:-/10	10/-:28,56/-		10/
4.		-/-:-/28		
6.	-/-:-/56	56/-:-/-		56/
7.		-/-:-/56,28,10		
10.	-/-:-/40,56	40,56/-:-/-		40/56
21.		-/-:-/40		
22.		-/-:-/56		
24.		-/-:81/-		
27.		-/-:-/56,28,10		
33.	-/-:-/5,110,81,10	-/-:-/81,28,10+		
35.	-/-:-/110,56,10	28/-:56,10/-+		28/
38.	-/-:-/56,10	-/-:-/40,56,10		
42.		-/-:-/56		
45.	-/-:56/-	56/-:-/-		56/
51.	-/-:-/40,70,10	-/-:-/40,70,10		
52.	-/-:-/5,110,70,56	-/-:-/70,56+		
56.	-/-:-/10	-/-:28,10/-		
58.	-/-:81/-	81/-:-/-		81/
61.		-/-:-/28		
64.	-/-:81/-	81/-:-/-		81/
68.		-/-:-/28		
70.		-/-:-/10		
73.		28/-:10/56		28/
74.		-/-:-/70		

TABLE X (contd.)

PHAGE TYPING OF LASSWADE ISOLATES.

Isolate no.	Method of Ruys and Borst (1959).		Method of the International Association of Microbiological Societies.	
	R.T.D.	100 x R.T.D.	R.T.D.	100 x R.T.D.
77.	-/-:-/110,70	70/-:-/-+		70/
79.		-/-:-/10		
83.	-/-:-/110,70	70/-:-/-+		70/
88.	-/-:81/-	81/-:-/-		81/
90.	-/-:-/70	70/-:-/-		70/
95.	-/-:-/56,28,10	28,56,10/-:-/-		28/56/10/
100.	-/-:-/28,10	28,10/-:56/-		28/10/
105.		28,10/-:56/-		28/10/
108.		10/-:28/56		10/
110.	-/-:5/-	-/-:-/81+		
114.	-/-:-/40,56	40,56/-:-/-		40/56/
116.	-/-:-/56,28,10	56,28,10/-:-/-		56/28/10/
120.	-/-:-/40	40/-:56/-		40/
121.	-/-:5/-	-/-:81/-+		
122.	-/-:-/5,81,56,28,10	56,28,10/-:-/81+		56/28/10/
127.	-/-:10/56,28	56,28,10/-:-/-		56/28/10/
129.		-/-:-/56,28,10		
130.	-/-:-/56,10	56,28,10/-:-/-		56/28/10/
131.		-/-:70/-		
132.	-/-:-/81	81/-:-/-		81/
134.	-/-:-/28,10	56,28,10/-:-/-		56/28/10/
135.	-/-:-/56,28,10	56,28,10/-:-/-		56/28/10/

+Phages 5 and 110 only used at R.T.D.

Fig. IV.

Phage typing.

Key to phages:

28^{10-3}	10^{10-1}	10^{10-3}	110^{N}	5^{N}
81^{10-3}	81^{10-5}	56^{10-3}	56^{10-5}	28^{10-1}
	40^{10-3}	40^{10-5}	70^{N}	70^{10-2}

a) Strain 58.

Read:

R.T.D. -/-:81/- or -

R.T.D. x 100. 81/-:-/-

or 81/.

b) Strain 64.

Read:

R.T.D. -/-:81/- or -

R.T.D. x 100. 81/-:-/-

or 81/.

c) Strain 73.

Read:

R.T.D. -

R.T.D. x 100. 28/-:10/56

or 28/.

d) Strain 77.

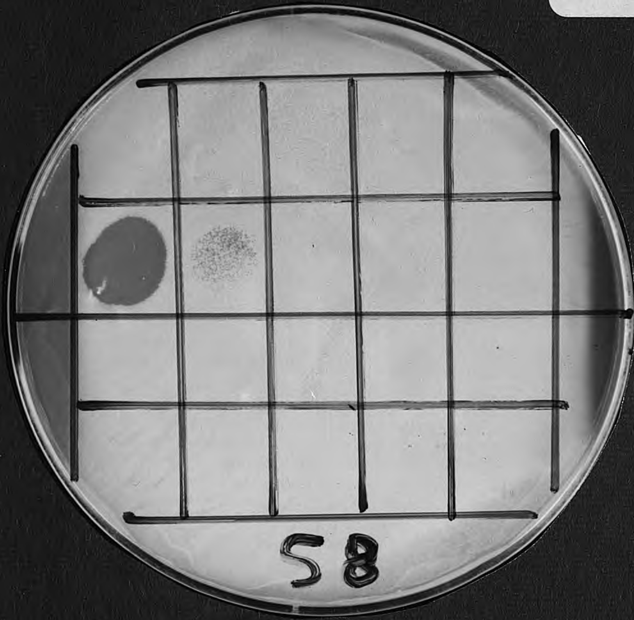
Read:

R.T.D. -/-:-/110,70 or -

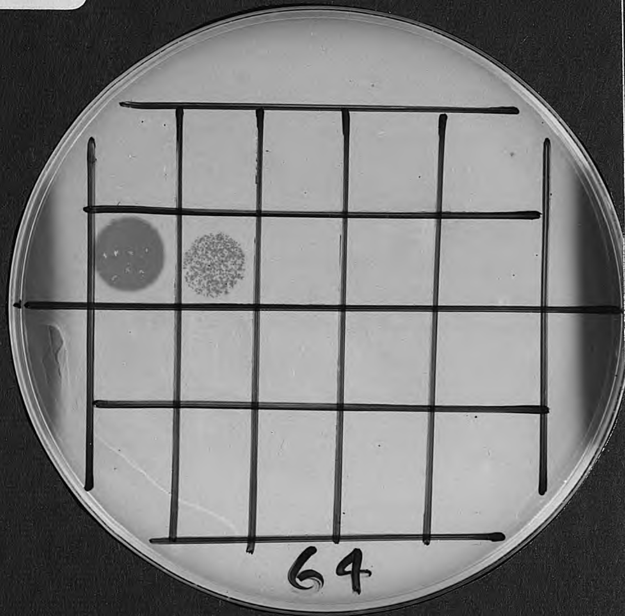
R.T.D. x 100. 70/-:-/-

or 70/.

FIG. IV.



a



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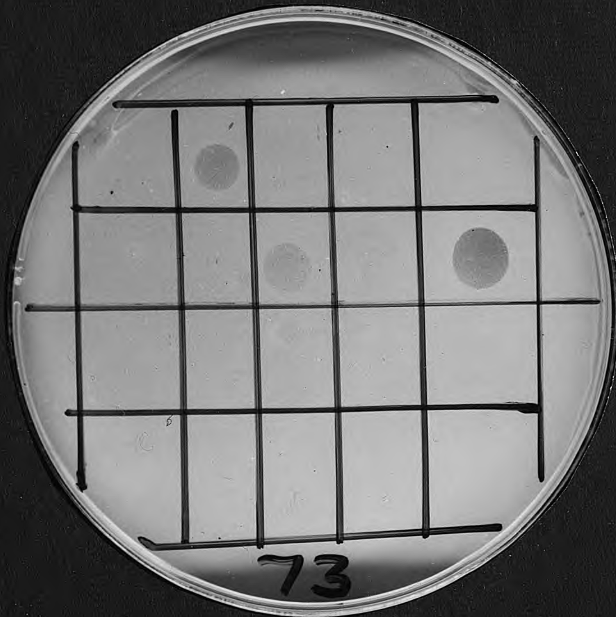


c

d

Fig. V.

Phage typing (contd.)

Key to phages:

10^{10-1}	10^{10-3}	110^{N}	5^{N}
56^{10-3}	56^{10-5}	28^{10-1}	28^{10-3}
70^{N}	70^{10-2}	81^{10-3}	81^{10-5}
		40^{10-3}	40^{10-5}

a) Strain 95.

Read:

R.T.D. -/-:-/56,28,10 or -

R.T.D. x 100. 28,56,10/-:-/- or 28/56/10/.

b) Strain 114.

Read:

R.T.D. -/-:-/40,56 or -

R.T.D. x 100. 40,56/-:-/- or 40/56/.

c) Strain 127.

Read:

R.T.D. -/-:-/56,28,10 or -

R.T.D. x 100. 56,28,10/-:-/- or 56/28/10.

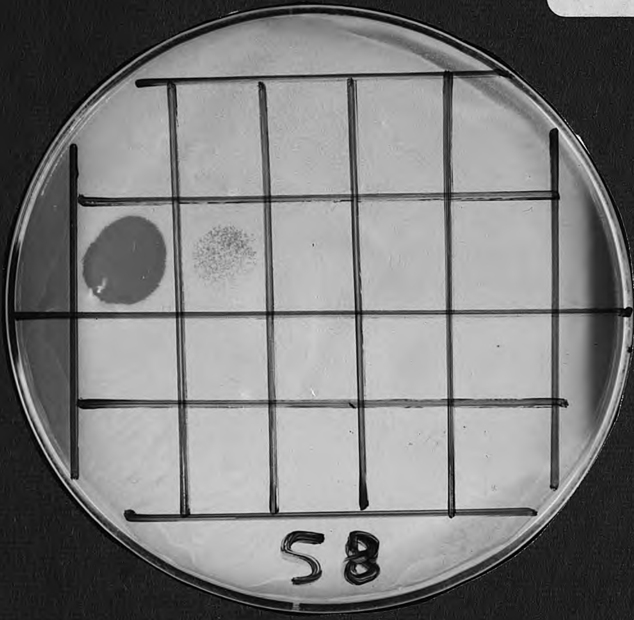
d) Strain 135.

Read:

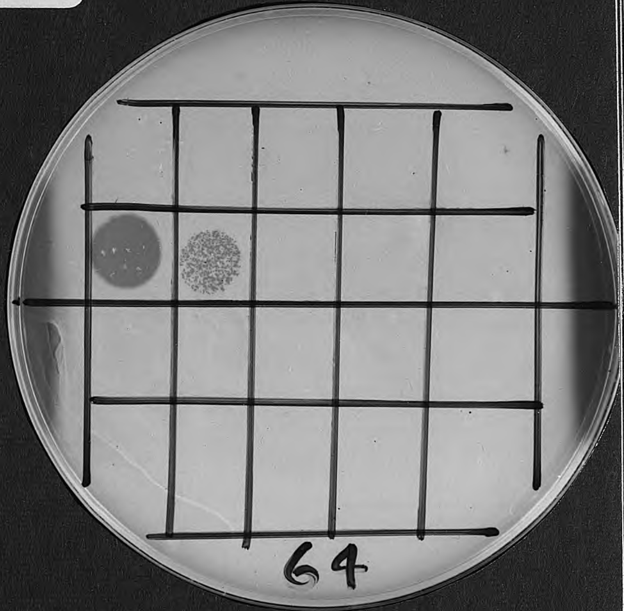
R.T.D. -/-:-/56,28,10 or -

R.T.D. x 100. 56,28,10/-:-/- or 56/28/10/.

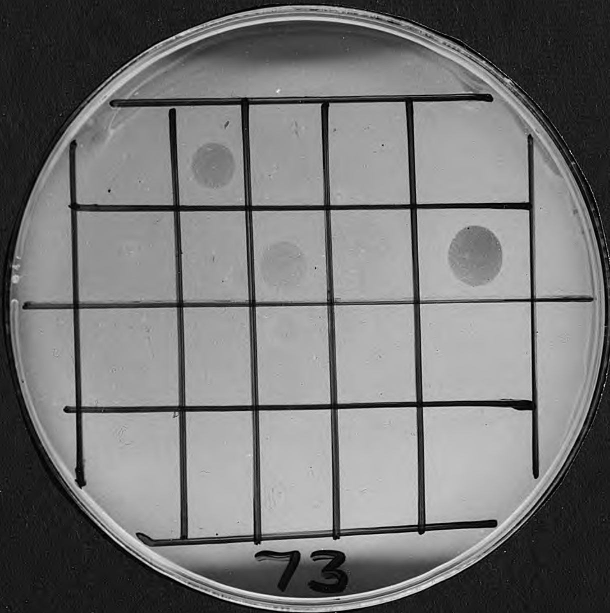
FIG. IV.



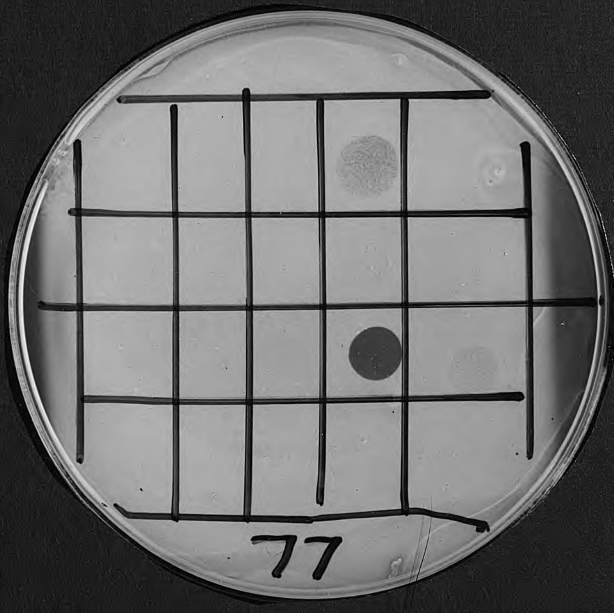
a



b



c



d

Fig. V.

Phage typing (contd.)

Key to phages:

10^{10-1}	10^{10-3}	110^{N}	5^{N}
56^{10-3}	56^{10-5}	28^{10-1}	28^{10-3}
70^{N}	70^{10-2}	81^{10-3}	81^{10-5}
		40^{10-3}	40^{10-5}

a) Strain 95.

Read:

R.T.D. -/-:-/56,28,10 or -

R.T.D. x 100. 28,56,10/-:-/- or

28/56/10/.

b) Strain 114.

Read:

R.T.D. -/-:-/40,56 or -

R.T.D. x 100. 40,56/-:-/-

or 40/56/.

c) Strain 127.

Read:

R.T.D. -/-:-/56,28,10 or -

R.T.D. x 100. 56,28,10/-:-/-

or 56/28/10.

d) Strain 135.

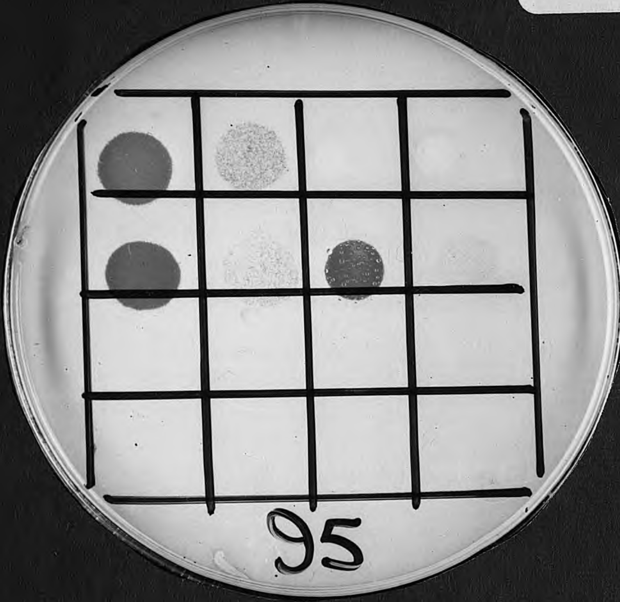
Read:

R.T.D. -/-:-/56,28,10 or -

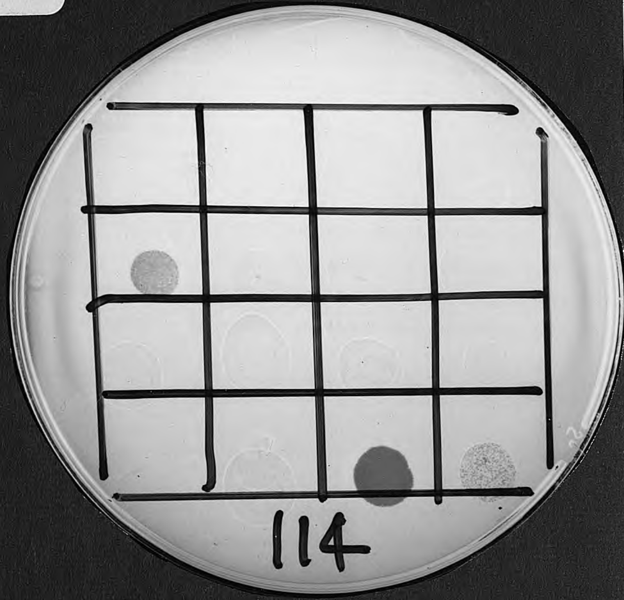
R.T.D. x 100. 56,28,10/-:-/-

or 56/28/10/.

FIG. V.



a



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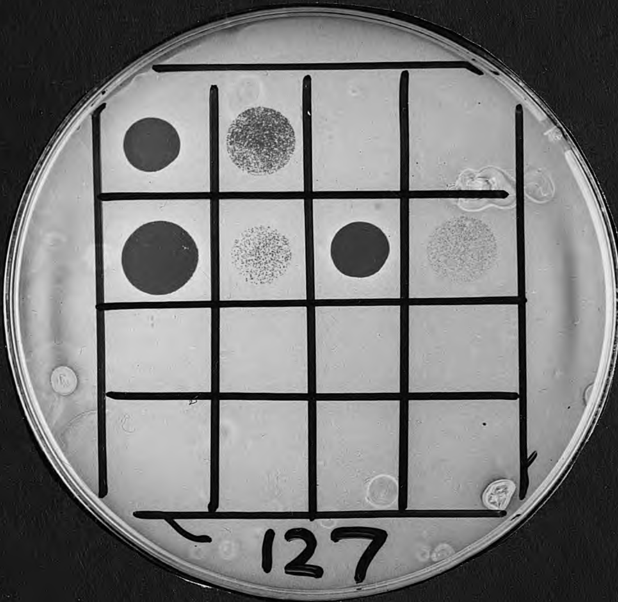

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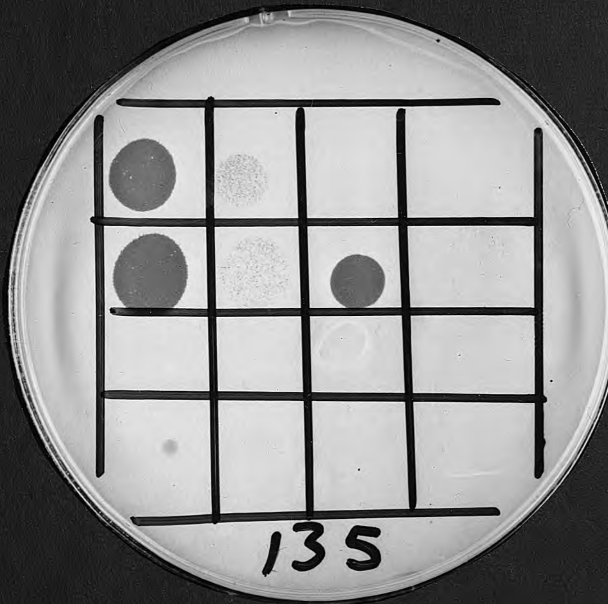


d

25 strains (21%) were phage typable using the method of the International Association of Microbiological Societies, and these were then divided into 5 groups, the members of each group having similar phage activity. This is shown in Table XI, together with the farm of origin of the strains within each phage group.

8 of the 13 strains in phage group A (61.5%), originated from farm H. The 2 strains in phage group B were from different farms (farms A and D).

2 of the 3 strains in phage group C originated from farm N.

3 of the 4 strains in phage group D originated from farm H.

2 of the 3 strains in phage group E originated from farm A.

TABLE XI.

Phage-typing of coagulase-positive staphylococci isolated at Lasswade

Phage group	Phage type	Sample no. (and farm of origin)
A	28/56/10	95(H),116(H),122(H),127(H),130(E),134(H),135(H)
	28	35(H), 73(H)
	28/10	100(D),105(E)
	10	1(A),108(B)
B	56	6(A), 45(D)
C	40/56	10(F), 114(N)
	40	120(N)
D	81	58(D),64(H),88(H),132(H)
E	70	77(A),83(A),90(owl)

The phage types on a particular farm were then related to their biotypes, as shown in Table XII.

This shows that, in general, the biotypes of any one phage group on a particular farm are usually varied. However, on farm E, the two strains typ able both fall into phage group A and biotype 7. Similarly, on farm A, the two strains that fall into phage group E belong to biotype C.

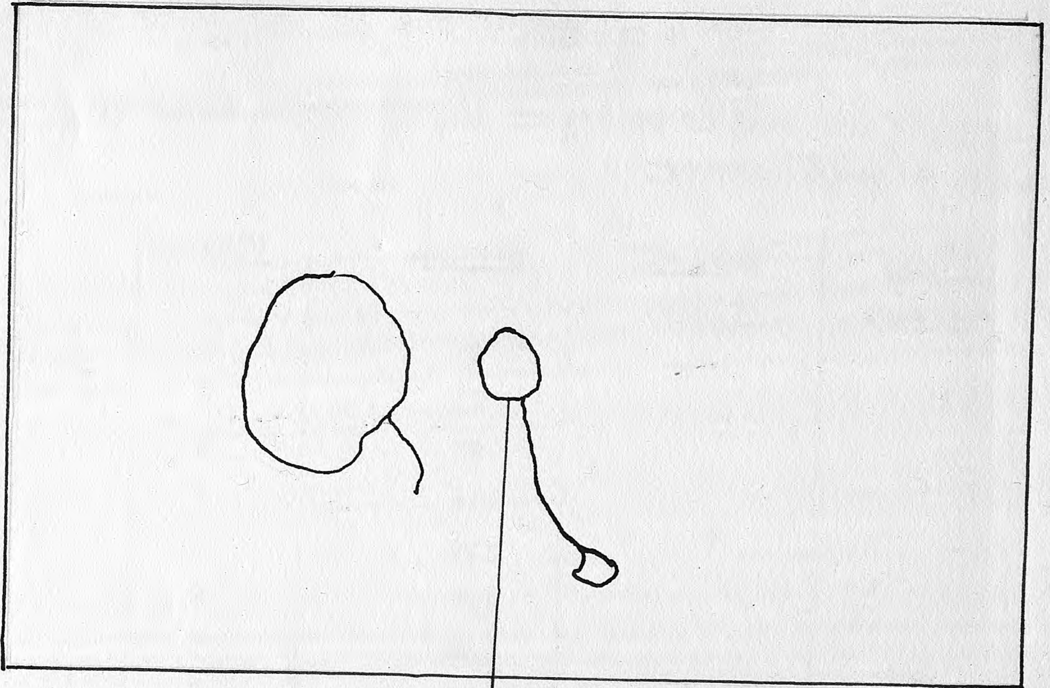
6. ELECTRON MICROSCOPY.

Electron micrographs of purified phage samples revealed a virus similar to that described by Smiles, Welch and Elford (1948). The phage head was round, with a diameter of 65 nm., the tail long and slender, with a length of 203 nm., ending in a small 'bob' with a diameter of 36 nm. (see Fig. VI).

TABLE XII.

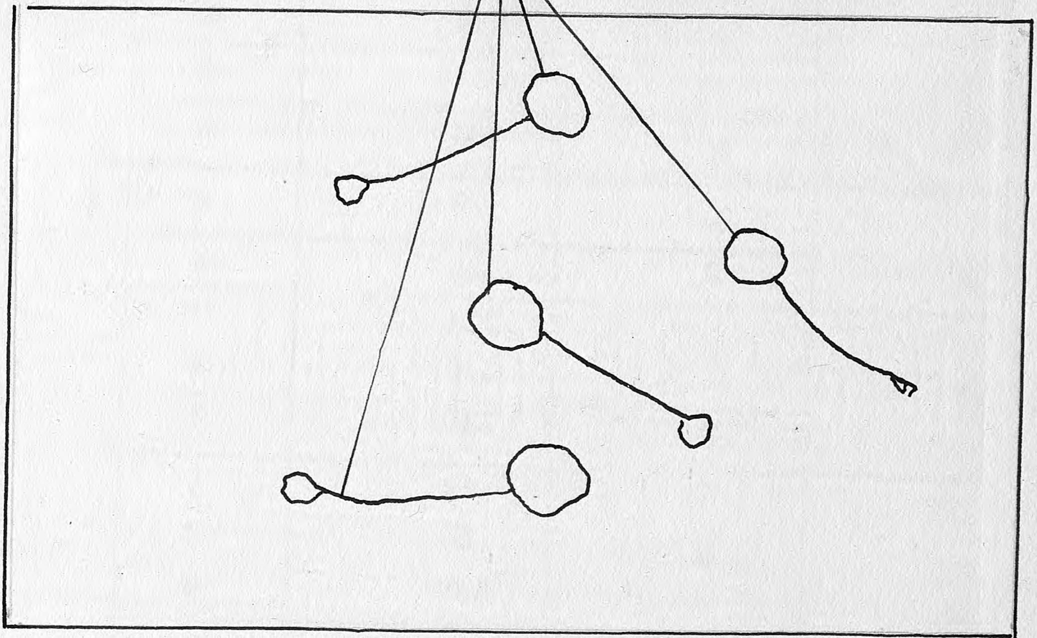
PHAGE GROUP AND FARM OF ORIGIN OF STAPHYLOCOCCAL ISOLATES, RELATED TO THEIR BIOTYPE.

Phage group.	Farm of origin.	Strain.	Biotype.
A	H	35	5
		73	6
		95	5
		116	7
		122	5
		127	4
		134	5
		135	7
	E	105	7
		130	7
	D	100	5
	A	1	5
	B	108	4
B	A	6	4
	D	45	4
C	N	114	7
		120	4
	F	110	4
D	H	64	5
		88	4
		132	4
	D	58	4
E	A	77	c
		83	c



(a)

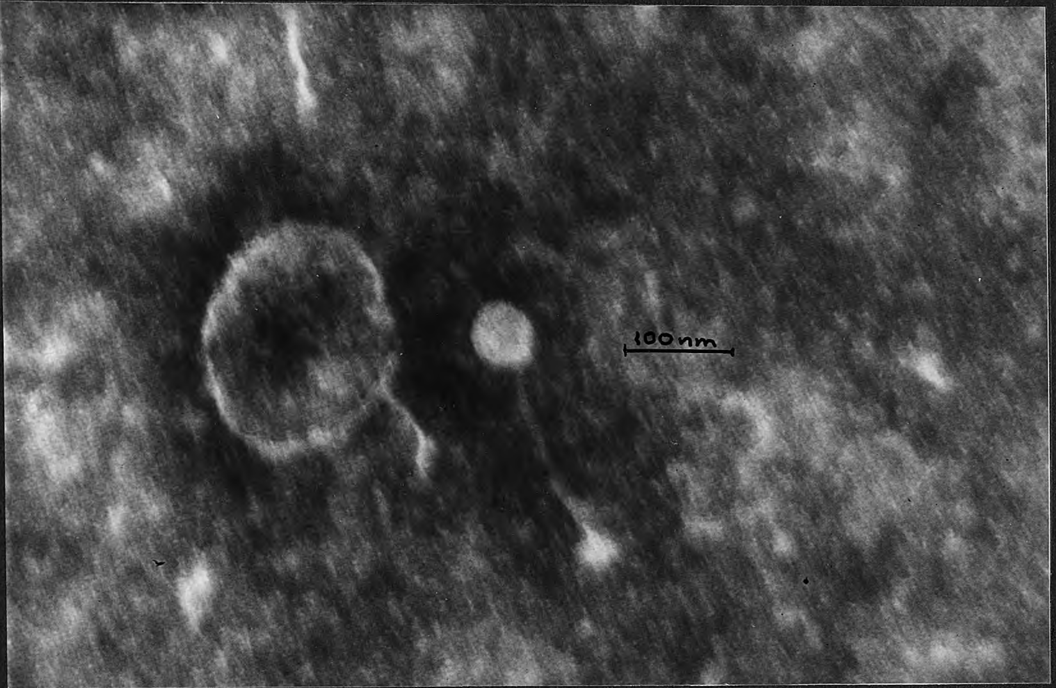
Phage virus particles.



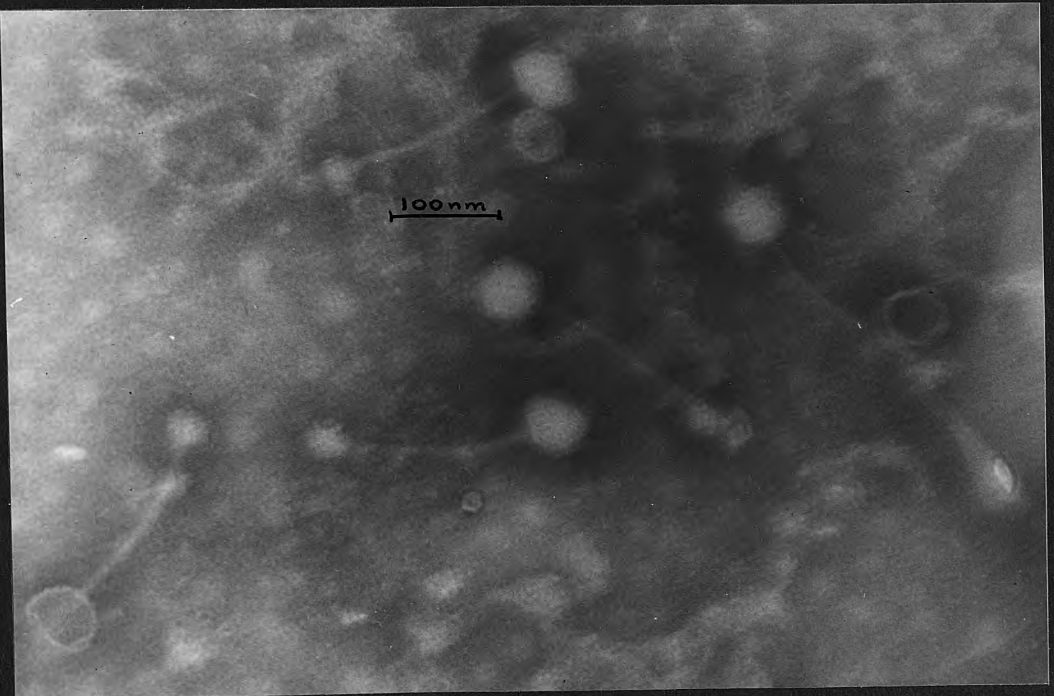
(b)

Magnification x 138,000.

FIG. VI.



a



b

DISCUSSION.

The biological characters examined were those used by Sato et al. (1972) to classify coagulase positive staphylococci into biotypes. The methods used to examine these characters were those of Sato, to enable a direct comparison to be made between the strains from Lasswade and those from Japan.

The results of the biological examination were similar to those of previous authors, and emphasised the difference previously noted between staphylococci of poultry origin, and those of human or other animal sources.

All the 119 samples coagulated rabbit plasma. 49 of the samples (41.2%) were haemolytic on sheep blood agar, whereas 70 (50.8%) were non-haemolytic. Smith (1947) noted that pathogenic staphylococci isolated from poultry often failed to produce haemolysis, unlike strains of human origin (Cowan, 1938). However, there is a higher incidence of non-haemolytic strains in the Lasswade isolates than in those studied by Sato et al. (1972), where 65 of the 1,021 strains gave no haemolysis. The mere presence or absence of haemolysis on sheep blood agar was recorded, in order that a direct comparison could be made with the work of Sato et al. (1972). A more detailed study of the type of haemolysin involved was not indicated in this investigation.

92 of the 119 strains (77.3%) produced yellow pigment, whereas 27 (22.7%) were white. This is in agreement with most earlier work, (e.g. Jungherr & Plastridge, 1941; Sato et al., 1972), and emphasises that although the majority of pathogenic strains are pigmented, a proportion is not. For this reason, the name *Staph. pyogenes* adopted by Fleming (1929) for the pathogenic strains of staphylococci would seem to be more appropriate than *Staph. aureus*.

100% of the Lasswade isolates were V-P negative, using the method of Barritt (1936). The control specimen, *E. aerogenes*,

gave a strong positive reaction. There are conflicting reports in the literature concerning the reaction of coagulase positive staphylococci to the V-P reaction. Cowan (1938) reported that 9 of the 13 coagulase positive strains of animal origin examined (69.2%) were positive to the test. Sato et al. (1972) found that 770 of the 1,021 coagulase positive strains of poultry origin (75.4%) were V-P negative. While some difference may exist in the reactions of staphylococci from different hosts, another factor may be involved. As previously stated, the methods for examining the biological characters of the Lasswade isolates were those of Sato et al. (1972), for reasons already explained. For the V-P reaction, the MRVP medium was inoculated with the sample under test, and incubated at 37° C. for 48 hours. However, Cowan & Steel (1965) found that incubation for 5 days at 30° C. was the minimum time required to detect all the positives among the enteric bacteria, using Barritt's method. For staphylococci, longer incubation for up to 10 days gave more positive results. This may explain why so many of the Lasswade and Japanese strains were V-P negative. In any future biotyping, it is suggested that the V-P reaction be tested after both 2 days and 10 days incubation of the sample.

The carbohydrates were fermented by the large majority of coagulase positive strains, and this is consistent with previous results (e.g. Smith, 1947; Kuramasu et al., 1967; Sato et al., 1972). 10 of the 119 strains (8.4%) failed to ferment galactose, and 3 strains (2.5%) failed to ferment lactose and mannitol.

With the biological reactions obtained, the isolates were typed.

No strains were classified as biotypes 1 or 3. One strain, no. 123, had the characters of biotype 2. However, it was considered that this strain was unique, and should not be classified as a biotype on its own, and it was reported as unclassified, type d.

38 strains were classified as biotype 4. Biotype 5 contained

47 strains, and these produced yellow pigment, fermented the carbohydrates, but failed to give haemolysis on sheep blood agar. 10 strains were classified as biotype 6, and 13 strains as biotype 7. Both these types produced no pigment, but differed in their ability to produce haemolysis.

These results clearly differ from those of Sato et al. (1972). The majority of the 1,021 strains tested by Sato belong to biotypes 1 and 3, while the majority of the Lasswade isolates belong to biotypes 4 and 5. Thus by this classification, the coagulase positive staphylococci isolated from poultry at Lasswade are biologically distinct from those isolated by Sato in Japan.

Two factors may have affected these results. Firstly, the variability of the characters used in biotyping. Numerous authors have reported variation of characters used in this study. Elek (1959), reviewing the subject, said it is well known that fermentation tests with staphylococci are unreliable and inconsistent, and even when a pure culture is plated, it is unusual for two colonies to give exactly the same biochemical tests. Concerning pigment variation, Elek reported that Courmont (1897) grew white and golden variants from the same abscess, and suggests that this variation merely reflects a more fundamental change. He added that the interpretation of pigment variation in terms of changes of the complex synthetic reactions involved, has not yet been attempted. Such colour variants cannot be detected by phage typing (Barber, 1955).

The second factor which may be involved is a fundamental biological difference between the staphylococci of poultry in Scotland and Japan. If this exists, the reasons for it could be numerous and complex, but may include such points of difference as breed of bird, genetic variation within a breed, age of bird affected, diet, management and antibiotic therapy.

It is more probable that the difference in results is dependant on a combination of the two factors mentioned above.

When the biotypes were related to the farms of origin of the strains, there was no correlation between a particular farm and a certain biotype, or a particular outbreak and a certain biotype.

The results of the present study would suggest that while there appears to be a fundamental biological difference between the strains isolated in Scotland and Japan, no such difference seems to exist between strains on different farms in Scotland. Furthermore, the outbreaks are not biologically distinct from each other.

4 screenings for phage, using the cross culture method, revealed 8 phages which were subsequently isolated and propagated. Sample 36 appeared to contain a phage lysogenic to all other strains. However, repeated attempts to propagate any phage failed. Examination of Fig. I reveals the area of lysis surrounding the 'spot' to be very broad. On the basis of this fact, and that a phage could not be propagated, it was concluded that the lysis was the result of some other lytic agent. The sample was abandoned as a potential source of phage.

The phage typing results indicate the critical nature of the reading of the R.T.D. titre. This was read as the highest dilution which just failed to give complete lysis (Blair & Williams, 1961). By this definition at least the 6 propagating strains should have been typable at R.T.D. Nevertheless, none were typable at this dilution, as the lysis produced was considered to be less than 50 plaques. This may have been due to a difference in thickness of the basal mat between the cultures used for titration and those used for phage typing. Also, a larger volume of phage was used in the titration than in the typing.

However, 25 strains (21%) were typable at 100 x R.T.D., including the 6 propagating strains. Since strains which show no lysis with the R.T.D. are usually retested with more concentrated phage preparations, Zierdt and Marsh have suggested that a single typing at 100 x R.T.D. would eliminate this second step and yield

a more reproducible phage pattern (Wentworth, 1963).

The author considers it advantageous to record results by the method of Ruys & Borst (1959) in addition to that of Blair & Williams (1961), as this facilitates grouping of phage types into phage groups.

In phage group A, 8 of the 13 strains (61.5%) originated from farm H. In groups C, D and E there also appeared to be a relationship between phage group and farm of origin. There was apparently no connexion between the phage group of an isolate, and its biotype.

These results must be interpreted with caution, and the following points should be considered.

1. 119 isolates were examined. This number could have been sufficient had all of them been phage typable, but as only 25 strains were typed, it is hard to draw any firm conclusions. Similarly, when those typable were related to their farm of origin, phage type and biotype, some correlation was present between phage group A and biotype 7 on farm E, and between phage group E and biotype C on farm A, but as only 2 strains were involved in each case, these results were not considered significant.

2. The set of phages selected by the Subcommittee on Phage Typing of Staphylococcus of the Nomenclature Committee of the International Association of Microbiological Societies for the examination of human strains consists of 21 basic phages. This represents years of continuous screening and selection of appropriate strains. Before a set of phages suitable for typing avian strains can be assembled, many more than the 8 phages detected in this study are required.

3. Although 61.5% of the strains in phage group 1 originated from farm H, many other samples on this farm, including some in the same outbreaks, were not typable. Whilst we might expect that if the staphylococcus has primary aetiological significance in poultry disease, the majority of strains isolated from a particular outbreak might belong to a single phage type, this need not necessarily hold.

4. The phage groups all contained strains of different geographical origin. It may be that these strains are primary pathogens, while those non-typable are secondary invaders. Other factors in the disease may need to be considered in relation to the phage typing of strains isolated, e.g. nature of lesion, breed of bird, feeding regimen, management proceedures, antibiotic therapy, age of bird affected and date of outbreak.

Previous work on the phage typing of avian staphylococci using the basic human phage set has shown poor results (Sato *et al*., 1961; Genigeorgis & Sadler, 1966; Harry, 1967; Kuramasu *et al*., 1967). In the typing of bovine staphylococci, considerable success has been achieved using a set of phages specific for bovine strains (Nakagawa, 1960; Davidson, 1961). The present study indicates that phages derived from avian strains could be of value in the typing of staphylococci from poultry. Future studies would require the search for and purification of further phages, so that the range of avian staphylococci susceptible to typing phages could be extended. This would ideally lead to the situation where the majority of staphylococci involved in any one outbreak could be typed by this method. Such a situation is essential if the correlation between phage type and epidemiology is to be accurately assessed.

CONCLUSIONS.

By the classification used in this study, the strains of coagulase positive staphylococci isolated from poultry at Lasswade appeared biologically distinct from those previously isolated by other workers in Japan. However, there was no such biological distinction between strains isolated from different farms or different outbreaks within Scotland.

In the phage typing, 21% of the strains were typable using a limited set of phages isolated from the strains themselves. This indicated that phages derived from avian isolates could be of value in the typing of staphylococci from poultry.

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