Verhandelingen

Studies on the content of haemagglutination inhibiting antibody for swine influenza virus A in sera from people living in the Netherlands 1957-1958

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STUDIES ON THE CONTENT OF HAEMAGGLUTINATION INHIBITING ANTIBODY FOR SWINE INFLUENZA VIRUS A

IN SERA FROM PEOPLE LIVING IN THE NETHERLANDS IN 1957–1958

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LII

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BY

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ERRATA

Page 4, second paragraph, line 2. Read: . . . . in the serum of 2 adults. . . .
Page 19, first paragraph, line 7. Read: . . . (it was higher in one person aged 12 and in 3 persons aged 16). At the ages 17–48 and one of 50, ...

INTRODUCTION

According to Dorset et al. (1922–1923), Dr J. S. Koen, an inspector in the Division of Hog Cholera Control of the Bureau of Animal industry, U.S.A., was the first to suggest that clinical and epidemiological evidence favoured the view that severe swine influenza was initiated by human cases of influenza during the 1918 pandemic. The disease was prevalent in swine during late August in Illinois and occurred amongst hogs in the Middle West during late summer and early autumn. Since that year, a similar epizootic has been regularly observed in swine in the U.S.A. as a seasonal phenomenon during late autumn and winter (see Shope, 1944).

The very important and often forgotten observation of Shope (1938) that in 1937 in 2 farms belonging to a prison and a State Home for Boys (U.S.A.) serological evidence was obtained that the human influenza virus A-PR8 had spread to droves of swine, indicates that Koen's observations from 1918 must be seriously considered.

In 1935, Laidlaw expressed the view that the strain type of swine influenza virus A, isolated for the first time by Shope in 1930 from swine in Iowa, might have been the cause of the human influenza pandemic of 1918 and that the study of the content of neutralizing antibody for the swine virus in sera from people of different age might produce further evidence for this hypothesis.

Studies on the presence of neutralizing antibody for swine influenza virus in comparison with that for human A strains were done by many investigators between 1935 and 1937. The results of these investigations, published by Andrewes et al. (1935), Shope (1936), Francis and Magill (1936) and Burnet and Lush (1938), are seen in fig. 1–4 [1]).

Although the techniques, the antigenic type of human strains used, and the number of sera investigated differ in these publications, the general outcome was much the same. Human sera collected in 1935 in England and the U.S.A., and in 1937 in Australia, generally showed that little or no neutralizing antibody for the swine virus was present before the age of 10–12 years (except for maternal antibody in the newborn). Above this age group antibody for the swine virus appeared, possibly comparable in amount to that for the human virus (see later). Shope (1936) emphasized the finding that amongst 123 sera which he investigated there were 17 with neutralizing antibody for the swine virus and none for the human strain A-PR8 (1934 U.S.A.). Combining this finding with the clinical, epidemiological and virological observations on swine influenza since 1918

[1]) Figures and tables are to be found at the end of this publication.

he concluded that: (1) antibody for swine influenza virus in man probably had originated in the 1918 pandemic; and (2) the influenza virus isolated in 1930 from North American swine probably is antigenically identical or closely related to the human and swine virus prevailing in 1918.

In a remarkable publication, Shope (1944) summarised the knowledge which existed concerning pandemic influenza at that time and re-asserted his earlier view, contrary to the doubts expressed by other investigators on the meaning of the presence of antibody for swine influenza virus in human sera. These doubts arose from the finding that swine influenza virus and human influenza virus A contain a different 'major' antigen but share 'minor' antigens. Antisera prepared in experimental animals with each type of virus showed in cross-neutralization tests high titred homologous antibody and also some antibody for the heterologous strain (Smith et al., 1935; Magill and Francis, 1936). This common antibody could be clearly demonstrated after repeated infection or vaccination of the same animal with either type of virus (Francis and Shope, 1936; Stuart Harris et al., 1938). From these experiments arose the argument that in man repeated clinical infection with strains of influenza virus might result in the appearance of heterologous antibody for the swine virus.

Also the investigations performed by Stuart Harris et al. (1938) on antibody for swine and human virus in sera collected in 1935 and 1936 from people living in St. Helena which had escaped the 1918 pandemic and had no outbreak of influenza during the years 1917–1921, furnished no definite proof as to whether or not swine antibody in man could possibly have originated in 1918 (see later).

After the discovery of the phenomenon of the haemagglutination induced by the influenza virus by Hirst, and McClelland and Hare in 1941, the antibody for strains of influenza virus in human sera could be measured by a simple and reasonably accurate in vitro quantitative titration. Moreover a few years later the outlook on the influenza A problem was broadened by the disappearance of the PR8-group of strains and by the appearance of an antigenic variant, which was first isolated in 1946 (the so called A1 group). This enabled the investigator to study the behaviour of antibody for a type of strain prevailing in the population during a definite known period of time.

Davenport and his associates, working in the laboratory of Professor Thomas Francis (School of Public Health, Ann Arbor, Mich., U.S.A.) attacked the problem on a large scale. In a series of very important studies published in the period 1953–1958 they obtained the following results.

(1) Pools of sera each containing aliquots from 25–55 individuals collected in 1952 showed a different age-pattern in the amount of haemagglutination inhibiting (H.I.) antibody, when examined against a strain of swine influenza virus (1931 U.S.A.) and human A, A1 and B strains (Davenport et al., 1953) (fig. 5). Basically the same patterns were found

in sera collected in England (Davenport et al., 1955) and Japan (Davenport and Hennessy, 1958). In 1952 demonstrable H.I. antibody for swine virus appeared in the serum pools of the 29 age group and showed high titres at the age of 35–36 years.

(2) Low levels of H.I. antibody titres for the human virus A-PR8 were found in a small percentage of individual sera collected from persons under the age of 10 and for the swine virus under the age of 20 (Hennessy et al., 1955).

(3) In studying the anamnestic H.I. antibody response induced by vaccination with monovalent influenza virus vaccines in people from different age groups, it was found that these responses varied with the age of the individual. After vaccination with A, A1 or swine virus, high heterologous antibody responses were found in children 4–10 years old only for the A1 strain; in persons 17–28 years old for both the A and A1 strains and in persons over 30 years old for the swine, A and A1 strains (Davenport and Hennessy, 1957; Hennessy and Davenport, 1958) (fig. 6).

The conclusions which they drew from their work, including previous observations, were as follows: (1) in human sera the age distribution and height of H.I. titres against human strains of influenza virus and the swine virus reflects the period of prevalence of strains of virus circulating in the community in the years after birth; (2) the antigenic 'blue-print' initiated by a major influenza virus antigen of the A-family can give rise, at a later period in life, to a strong anamnestic production of antibody for that antigen after vaccination (or infection) with heterologous human A and A1 strains or with the swine virus; (3) high titres of H.I. antibody for the swine virus in persons over 30 years of age (1952) must have originated in a short period starting around 1918; (4) such high titres of H.I. antibody for swine virus in people over 30 years of age may have been produced in part by an anamnestic reinforcement by infections with A and A1 strains which appeared later than the pandemic of 1918; (5) the major antigen of the swine influenza virus, isolated from swine in 1930–'31, must be identical or closely related to that of the virus which caused the human pandemic of 1918.

When pandemic Asian (A2) influenza appeared in the United States in 1957, Shope (1958) again reviewed briefly the virological problem of pandemic influenza. He compared the results of the investigations of the Ann Arbor group of workers on human sera in 1952 with his own from 1935. In his comparison of both sets of investigations he pointed out the difference in age-distribution of antibody for swine influenza virus in the two years and to the approximately identical time-relationship which they indicated for the age of appearance of swine antibody of high titre in man, namely the year 1918.

The theory of Shope and Davenport and co-workers found quite unexpected and important support by the experiments done by Jensen

and associates (1956), also working in the School of Public Health, Ann Arbor.

As early as 1938 Burnet and Lush had observed that both the antibody for the swine virus and for the human influenza virus A in the serum of an adult could be absorbed by the swine influenza virus. In contradistinction, antibody for the human virus in the serum of a child retained its full titre after absorption of the serum with the swine antigen. Jensen and co-workers rediscovered this phenomenon. They infected ferrets successively with 3 strains of the human A family (A-WS (1933), A-Weiss (A-PR8 type, 1943) and A1-Cam (A1 type, 1946)) at the respective intervals of 7 months and 6 weeks. All antibody in the sera could then be removed by absorption with the strain used for the first infection (A-WS). However, absorption with both other antigens removed all the homologous antibody in each case but only a limited amount of the heterologous antibody. Applying this method to human sera from persons in different age groups they found the same phenomenon described by Burnet and Lush. In sera from persons older than 30 years, swine influenza virus absorbed not only the homologous antibody but also all antibody present for the A and A1 strains. It failed to remove this antibody present in the sera from the age groups 17–26 and 4–10. From these and other experiments with human sera, obtained after vaccination, they concluded that: 'absorption of sera from groups of persons, both normal and after vaccination, results in complete removal of antibody for all strains of influenza virus within a type, when a strain of antigenic composition similar to that presumed to be the strain of first experience was employed.'

So far as we know, there is no explanation for this peculiar serological phenomenon but it does support the theory of Shope and Davenport and co-workers which stated that around 1918 human infections occurred with a strain of influenza virus which contained the major antigen of the swine influenza virus A.

Present study. It was the aim of this study to investigate the age distribution of H.I. antibody for swine influenza virus A and for strains of human influenza virus A and A1 in sera from people living in the Netherlands and also to compare the findings with the results obtained by earlier as well as recent workers on the same subject. We hoped to obtain more precise information relating to the problem of the origin of H.I. antibody for this virus in man by examining individual sera and by employing a strain of swine influenza virus of high avidity for antibody. It was also felt that an 'influenza virologist' should examine, at some time in his career, the outstanding problem in influenza virus archeology of the pandemic year 1918 and have the opportunity to repeat some of the highly important and basic work, done by the original workers. The circumstance that a classic pandemic of influenza re-appeared in 1957, which was caused by a new family of influenza virus A, furthermore made it possible to obtain a broader outlook on the virological and epidemiological problems of pandemic influenza in general.

MATERIALS AND METHODS

Human sera. Before the spread of A2-influenza in the Netherlands (June 1957), 1256 specimens of human sera of different age groups had been collected; in the autumn of 1958 another 3091 specimens were obtained (about 40 sera per age group).

Ferret-antisera. Anesthetized ferrets, 8—20 months old, are infected intranasally with 1 ml of virus-containing allantoic fluid (0.5 ml per nostril). After 2—3 weeks the animals are bled. Each pre-infection serum is checked for the presence of antibody for influenza virus by using strains which had been regularly passed in our laboratory. Inoculated ferrets are isolated rigidly. The human and ferret sera are stored at —18° C.

Vaccines. The preparation of adjuvant vaccine is done with allantoic fluid. This is concentrated in a high-speed centrifuge (1½ hour at 16,000 RPM). The concentrated virus titre is about 8,000. In 10 parts allantoic fluid we use 0.5 parts Arlacel and 9.5 parts Bayol F. Occasionally a culture of Mycobacterium butyricum is also added to the vaccine. The vaccines employed in the human vaccinations were kindly supplied to us by the Laboratories of the N.V. Philips-Duphar, Weesp, Netherlands.

Strains of influenza virus. Most strains of swine influenza virus were obtained from Dr R. E. Shope (Rockefeller Institute for Medical Research, New York). We also obtained from Dr R. Q. Robinson (at that time working in the Communicable Disease Centre, Virus and Rickettsia Section, Montgomery, Alabama) a strain isolated from swine in 1957.

The swine strains used, were: A-swine-15 (1930 U.S.A., Iowa) $F(erret)_x$ $M(ouse)_x$ $E(gg)_{57-88}$ and $F_xM_xE_{55}M_6$ E_{2-5}; A-Swine-15 (1930 U.S.A., Iowa) $M_{257}E_{2-5}$; A-Swine-23 (1935 U.S.A., Ohio) $M_{75}E_{2-3}$; A-Swine-29 (1937 U.S.A., Iowa) $M_{68}E_{5-6}$; A-Swine-30 (1940 U.S.A., Iowa) $M_{34}E_{2-3}$; A-Swine (1946 U.S.A.) $M_{21}E_2$ ('Vogt'-strain); A-Swine (1957 U.S.A., Wisconsin) E_{3-8} and $E_9M_{15}E_1$. A strain of horse influenza virus A-1742 (1956 Praha) was obtained from Dr O. Fiserova-Sovinova, Prague, Czechoslovakia. The passage lines employed were E_{10-12} and $E_{10}M_{30}E_3$. The human strains used, were: A-WS (1933 England) $F_xM_xE_{89-91}$; A-PR8 (1934 U.S.A.) $F_{198}M_{593}E_{55}M_3E_{63-66}$; A1 (1947 England) E_xE_{30} and $E_xE_1M_{20}E_5$; A1 (1949 Nederland) E_{49} and $E_8M_{20}E_9$ and $E_8M_{20}E_8M_5E_4$; A1 (1951 England, Liverpool type) E_xE_5 and $E_xE_6M_{51}E_4$ and $E_xE_6M_{51}E_3M_5E_4$; A1 (1951 Nederland, Scandinavian type) E_{36} and $E_6F_{10}E_8M_{18}E_6$; A1 (1953 Nederland) E_{44} and $E_8M_{17}E_{4-11}$; A1 (1956 Nederland) E_8 and $E_5M_{23}E_{5-8}$; A2-305 (1957 Japan) $E_5F_3M_6E_8$.

Adaptation of egg-line strains to mice. Anesthetized mice are infected intranasally with 0.05 ml of 10% emulsion of infective lung tissue. Serial passage is continued until all the infected mice die spontaneously of typical influenza virus pneumonia within 10 days.

Passage of strains. The egg-line strains and also the mouse-adapted lines

are passed by inoculation into the allantoic cavity of 10 or 11 days old chicken embryos. To prevent bacterial growth, penicillin (500 U/ml), streptomycin (500 μg/ml), and sulfamethylpyrimidine (200 mg%) are added. Test-pools of infected allantoic fluid, diluted 1 : 4 in merthiolate (0.01%) are used in the H.I. tests.

The preparation of Vibrio cholerae filtrate for the removal of non-specific inhibitor present in human and ferret sera. A strain of Vibrio cholerae (4Z, obtained from Sir Macfarlane Burnet) is passed every 3 weeks on nutrient agar (containing 2% Caseine peptone, Merck, and 1.5% Bacto Agar, Difco) and stored at 4° C. Vibrio cholerae is inoculated in pre-seed broth, which is then incubated for 8 hours at 37° C. The pre-seed culture medium contains 0.5% NaCl, 2% peptone SS (Brunnengräber) and 7.5 ml Na_2CO_3 20%, per 1000 ml broth, and is adjusted to pH 6.9—7.0. The pre-seed broth is then used to prepare the nutrient agar (0.6% agar).

The incubated pre-seed broth is spread on the surface of the nutrient agar in metal containers (30 × 19 cm) and incubated 16 hours at 37° C. The medium is then twice frozen at —20° C. and thawed after each time. The fluid is pipetted and then filtered through Carlson filters (pressure not higher than between 10 and 20 cm Hg!). Generally the pH of the filtrate is between 7.16 and 7.45 and should not exceed 7.6. The filtrate is divided into small quantities which are tested for contamination and finally stored at 4° C. The peptone SS is divided into portions of 20 grams and sealed in vacuo.

Testing the potency of the filtrate. One part of normal ferret or rabbit serum known to contain a large amount of nonspecific inhibitor, is treated with 2 and also 5 parts of the filtrate. Also a human serum without antibody for the strain A2-305 (1957 Japan) $E_5F_3M_6E_n$ is included in the experiment. After 16 hours of incubation at 37° C. the tubes are then placed at 56° C. for one hour.

The test strains employed, are A (1941 Nederland) (ferret-mouse-egg-line), A1 (1947 England) (egg-line) and A2-305 (1957 Japan) (egg-ferret-mouse-egg-line). The strain A (1941 Nederland) and A2-305 (1957 Japan) are highly sensitive to 'alpha' inhibitor and the strain A1 (1947 England) to 'beta' inhibitor. In all tests no demonstrable non-specific inhibition should occur in the lowest dilution of serum. From time to time it is necessary to replace the passage culture of Vibrio cholerae in use with a new freeze-dried specimen.

In all our investigations 5 parts of the filtrate are used. A normal ferret serum treated with the same filtrate is included in every experiment.

Chicken erythrocytes. Chicken red blood cells are obtained by heart puncture from anesthetized chickens. The blood is aspirated into a syringe containing Na-citrate, washed 3 times with saline and then stored as a 10% suspension at 4° C. The erythrocytes are used within 3 days after collection.

Haemagglutination inhibition test. This test is performed by means of a micro-method (Van der Veen and Mulder, 1950). A series containing 2 drops of 2-fold serum dilutions in saline is mixed with one drop of virus (2½—4 A.U.). After standing for half an hour at 4° C. one drop of a 2% chicken red-cell suspension is added. After half an hour the patterns are read. The figures obtained are then theoretically corrected to 50% haemagglutination and for the use of 3 A.U. of virus.

Absorption tests. Human sera were inactivated for 30 minutes at 56° C. to prevent haemolysis.

To 1 ml of packed chicken red cells, 6 ml of virus containing allantoic-fluid is added and the cells resuspended. The mixture is then kept at 4° C. for 30 minutes. Next the red cells are centrifuged and the supernatant fluid removed. Due to the rapid action it proved unnecessary to centrifuge at 4° C. After centrifugation the above procedure is again repeated. 1 ml human serum is added to 1 ml treated packed red cells and incubated for 2 hours in a 37° C. waterbath. The mixture is repeatedly shaken at intervals of 10 minutes. After centrifugation this procedure is also repeated again. Then the serum is treated 2—4 times with 0.05 ml of packed chicken red cells to remove any free virus present.

A parallel test is performed with the same serum which is mixed with untreated chicken red cells.

CRITICISM OF THE METHODS USED

The use of crude cholera filtrate for the removal of serum inhibitors. After the publications of Mulder and Van der Veen (1948), and Van der Veen and Mulder (1950) on the removal of serum inhibitors of influenza virus by the application of a crude cholera filtrate, several investigators confirmed the impression of these authors that the amount of specific antibody is not reduced by the filtrate (Appleby and Stuart Harris, 1950; Tyrrell and Horsfall, 1952; Hilleman and Werner, 1953). Occasionally however, it has been found that a reduction of specific antibody may occur (Isaacs and Bozzo, 1951; Hilleman et al., 1958). For this reason a re-examination of this question was found necessary. The tables 1 and 2 show the investigations done. The conclusion which can be drawn from the experiments is that a crude cholera filtrate, as it is prepared in our laboratory, does not reduce the H.I. titre of specific antibody for the strains of virus employed. It is extremely probable that this will also be the case with any other antiserum. Duplicate tests done with cholera filtrate and potassium periodate-treated sera generally showed that the same titres of antibody were present. Using the periodate method, in every modification which has been recommended, we were unable to remove all the serum inhibitors of the 'alpha' type in a small percentage of human sera which had been stored at −18° C. for a few months.

The possibility of non-specific inhibition in the H.I. test caused by antibody for egg-protein (Knight, 1944; Harboe et al., 1961). In order to examine this possibility 9 ferrets were infected intranasally with normal allantoic fluid from 12–13 days old chick embryos. This was repeated 5 times. The ferret sera were then examined for the presence of H.I. antibody using different purified strains of influenza virus A and B. The tests were done using the same methods employed in the routine H.I. tests, when using influenza virus antiserum of ferrets. No such antibody for egg-protein could be demonstrated. Ten ferrets were vaccinated with adjuvant vaccine containing normal allantoic fluid. The resulting sera did not show any measurable H.I. antibody for different strains of influenza virus.

The accuracy of the H.I. test. The accuracy of the H.I. test as it is performed in our laboratory is reasonably high, but for crucial studies the tests should be done in duplicate and should also be repeated one or two times.

For the comparison of the titres in selected sera from different age groups and against one particular strain, the tests should be done with the

same materials on the same day. H.I. tests performed on different days may only give a tentative result as far as the height of the titres is concerned. However, these are suitable as a screening method for the selection of sera with high or low titres.

The avidity of strains of influenza virus for antibody. The avidity of strains of influenza virus for antibody is an ever lasting problem of insecurity to the investigator. Table 3 shows the difference in avidity for antibody of two laboratory strains of swine influenza virus. Since we received the most avid strain A-Swine-15 (1930 U.S.A.), with the passage formula Swine-Mouse$_{257}$, from Dr R. E. Shope in the midst of our work, we considered it necessary, with the exception of one experiment, to repeat all investigations done so far with the less avid strain of swine virus.

In comparing the height of H.I. titres present in one particular serum against different strains, all tests should be done on the same day. Theoretically speaking the strains used in the experiment should have an optimal avidity for antibody. Therefore all strains should be 'P'-derivatives (Van der Veen and Mulder, 1950; Choppin and Tamm, 1960). To ensure this, strains isolated in embryonated eggs were adapted to mice by intranasal inoculation.

To obtain some insight into the avidity for antibody we passed the human A and A1 strains, which were used in our experiments, 4 times at limiting dilution in eggs (Choppin and Tamm, 1960). We then compared the avidity of the parent strains for homologous antibody with that of the strains obtained in the highest dilution from the final passage. No differences were observed.

Despite these precautions however we have no definite proof, that all strains used in our experiments were of optimal avidity for antibody. Therefore the height of the H.I. titres present against these strains in a particular antiserum is only of relative importance and a comparison of the height of these titres can only be based on the balance of probabilities.

The problem of avidity for antibody is of particular importance when low titred antisera are used. Experience with the A2 type of influenza virus has taught us that a certain strain may show a good avidity when the H.I. titre of the antiserum is high but cannot demonstrate H.I. antibody in a low titre. Therefore it is necessary that with each strain preliminary experiments should be done in which 2-fold serial dilutions of the homologous antiserum are made in normal serum. Only when the highest dilutions of H.I. antibody are demonstrated can the strain be considered suitable for use in the experiments (table 4).

The choice of strains of influenza virus of a certain antigenic composition to be used in the experiments. This choice is inevitably arbitrary but may influence the outcome of the investigations. Therefore the antibody patterns in relation to age found, using certain strains of virus,

depend upon the particular antigenic composition of these strains and do not necessarily correspond to patterns of antibodies for other isolates of the same sub-group.

Differences in antigenicity between strains of influenza virus. Differences in human antigenicity between strains of influenza virus do exist. Partly antigenicity is related to the human virulence of the strains. The antigenicity of the different strains used has not been compared carefully in this study, but it is almost certain that the antigenicity of the swine influenza virus A is higher compared with the other strains used.

The individual antibody response of ferrets after infection or vaccination with strains of influenza virus. Different ferrets may show different responses to minor antigens which are shared between different strains of influenza virus used in the experiments. Therefore in investigating relationships between strains of influenza virus one never should rely on a single ferret antiserum alone.

The possibility of contamination of laboratory strains of influenza virus. Every worker in influenza virology is aware of the possibility that strains of influenza virus may be contaminated in the laboratory and that there is always uncertainty as to whether or not the lines used are pure. Therefore it is important that results of cross-tests obtained in one laboratory should be checked at some time by other laboratories in order to reduce the possibility that wrong conclusions may be drawn from the experiments.

The unreliability of the age of the serum donors. Since we were not able to obtain a sufficient number of sera from different age groups in the Medical Clinic we had to rely on the ages which were registered on the label of the tubes sent to us. In the course of the investigations we found on serological grounds, that in some cases mistakes in the labelling had been made (see later), but is was impossible to check the age of each person given.

The antigenic relationship between strains of swine influenza virus A isolated in the period 1930–1957 in the U.S.A. Gompels (1953) and Jensen and Peterson (1957) compared the antigenic composition of different swine strains isolated in the period 1930–1954 and came to the conclusion that no great antigenic differences in those isolated in the U.S.A. were demonstrated. Table 5 shows the H.I. cross-tests done in our laboratory using different swine strains (1930–1957) from the U.S.A. There is no doubt that the strains investigated can be placed in one antigenic group, even though there are differences which are of the same order as those occasionally observed between human strains of influenza virus A, A1 or B which were isolated in the same epidemic.

Minor antigens shared between swine influenza virus A and human strains. Many investigators have stated that, at present, influenza viruses A can be antigenically divided into 'sets' or groups of strains, e.g. swine A, human A, A1 and A2. However, the impression might arise from such a division that these 'sets' are more or less separate groups and that the sharing of minor antigens between these groups is of secondary importance. Therefore we re-investigated carefully the minor antigens shared between strains of swine influenza virus and human A, A1 and A2 strains employing the H.I. test. In all experiments a certain number of animals should be used in order to eliminate differences in individual antibody responses.

Sharing of minor antigens between strains of influenza virus can be demonstrated in different ways: (1) by applying the cross H.I. test using animal antisera obtained after intranasal infection or parenteral injection; (2) by vaccination of animals with adjuvant vaccines; (3) by successive intranasal infection or parenteral injection of animals with the same or different antigenic types of human A strains and using appropriate time intervals; (4) by initiating in animals an antigenic 'blue-print' by using a certain strain and then re-infecting the same animals with another antigenic type of strain when the amount of antibody has declined (re-infection test; see also Harboe, 1960). A parallel type of experiment is that of vaccinating persons of different age with monovalent vaccines of strains of swine influenza virus and human strains and then to investigate the rise in antibody titres against the homologous and heterologous strains (see introduction) or to determine the heterologous anamnestic recall of titres after natural infection with a certain antigenic type of strain.

The fourth type of test may be the most sensitive in use at present, but the interpretation may be hampered by the constantly existing possibility of contamination of the strains employed. Another doubt concerning the reliability of this kind of test is that the underlying mechanism which determines the results is not fully understood. Unknown host factors in particular may interfere with the results, thus necessitating the use of a rather large number of animals or humans in each experiment.

Cross-relationship between strains of swine influenza virus and human A and A1 strains, using the H.I. test. Table 6 shows that ferret antisera against human A and A1 strains prepared with adjuvant vaccine contain more cross-inhibiting antibody for the swine virus than those prepared by intranasal infection. The same is true for antisera against swine influenza virus (table 7). Tables 6 and 7 show that cross-reactions between the swine and human strains can be demonstrated inconstantly and are found to occur with different human A and A1 strains. A few heterologous titres were nevertheless high. From these experiments it is clear, that an antigenic relationship between swine influenza virus and human strains of influenza virus A and A1 can be demonstrated in the cross H.I. test. An interesting observation is that in the cross H.I. tests different but closely related laboratory lines of swine influenza virus react differently.

Antigenic relationship between the swine virus and the first isolated human strains of influenza virus A-WS (1933 England) and A-PR8 (1934 U.S.A). From the table 7 it can been seen clearly that many antisera against different swine strains, which were prepared by intranasal infection or by vaccination with adjuvant vaccines, show distinct cross reactions with the human strain A-PR8 (1934 U.S.A.), but in only one instance with the strain A-WS (1933 England) and only occasionally with A1 type strains. However, in 1949 we found that reciprocal cross reactions between our laboratory line of swine virus and the strain A-WS were present in the cross H.I. test (titres 30 and 45 respectively). In 1954, they could not be demonstrated again when employing the same line of strains. In earlier experiments using re-infection tests, or cross neutralization tests in animals, an antigenic relationship between the swine virus and the human strains of the A-WS and A-PR8 type could be demonstrated (Smith et al., 1935; Magill and Francis, 1936; Magill and Francis, 1938). It is therefore possible that by long continued passage of influenza viruses in eggs under certain circumstances variants were selected which for the greater part had lost minor antigens shared between heterologous strains. However, it should be noted that the results obtained with re-infection tests are not quite comparable to those obtained by cross neutralization (or cross H.I.) tests (Oakley and Warrack, 1940).

The appearance of H.I. antibody for swine influenza virus induced by successive intranasal infection of ferrets with human A and A1 strains. The tables 8, 9 and 10 show the appearance of H.I. antibody for swine

virus after repeated intranasal infection of ferrets with human A and A1 strains at intervals of 3 weeks. In these experiments antibody for swine virus appeared without exception but they demonstrate again the differences in individual response. Generally speaking, the titres against the swine virus were low but in 2 ferrets high titres did appear.

Cross re-infection experiments in ferrets, employing the swine virus and human A and A1 strains. In all experiments the second infection was initiated when the amount of H.I. antibody induced by the first infection had declined substantially.

Table 11 shows the results of these experiments. With 2 exceptions all ferrets showed a high antibody response to the dominant antigen of the primary infecting strain, which was irrespective of the antigen used either for the primary or secondary infection.

Cross tests with the strain of swine influenza virus, human A, A1 and A2 strains. By including the A2 strain A2-305 (1957 Japan) $E_5F_3M_6E_8$ in the above-described types of experiments the following results were obtained.

(1) When antisera obtained by intranasal infection were used no cross reactions in the H.I. tests, in a dilution of > 9, could be observed between the A2 strain, the human A and A1 strains, and the swine virus.

(2) Only one of seven antisera against swine virus, prepared with adjuvant vaccine, showed low titres (< 100) with different A2 strains used. However, none of the antisera against human A or A1 strains, prepared in the same way, crossed with the A2 virus in a dilution of > 9. Antisera against the A2 strain, also prepared with adjuvant vaccine, did not cross with swine virus, human A and A1 strains.

(3) Six ferrets vaccinated with an adjuvant vaccine containing a mixture of different human A and A1 strains did not develop demonstrable H.I. antibody for the A2 virus but 4 of them did develop antibody for the strain of swine influenza virus (table 12).

(4) After repeated intranasal infection of ferrets with the swine virus and (or) human A and A1 strains no demonstrable H.I. antibody for the A2 virus appeared (table 8, 9 and 13).

(5) Cross re-infection experiments in ferrets with the human A, A1 and A2 virus and the swine virus showed less constant recall of titres to these strains than those performed with the swine virus and human A and A1 strains (table 14).

Cross tests including the strain of horse influenza virus A. In preliminary experiments which included the horse influenza virus A, no cross reactions could be observed either in the H.I. tests, or by applying repeated intranasal infection.

In cross re-infection experiments very few ferrets showed an anamnestic recall of titres (table 15). Curiously enough these were most clearly present in cross experiments using the strain A1 (1956 Nederland). No

H.I. antibody for the horse virus could be found in human sera of different age groups.

Conclusion. From the above described investigations it can be concluded that, so far as sharing of minor antigen is concerned, the antigenic 'distance' demonstrable between the swine influenza virus and the human A and A1 strains is much less than that between these three types of strains and the human A2 virus, and still much less than that between these three types of strains and the horse virus. This can be expressed in the following figure.

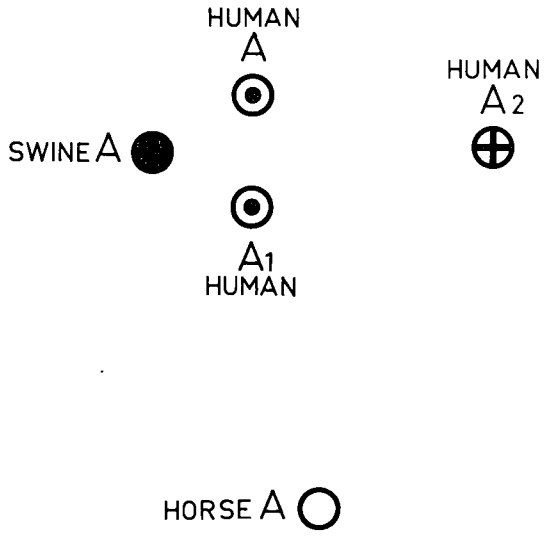

Figure 7. Diagramatic drawing showing the relative antigenic distances between 5 strains of influenza virus 'A'.

The content of H.I. antibody for strains of human influenza virus A and A1 in individual sera of different age groups. In studying the content of H.I. antibody for strains of influenza virus in human sera, both the percentage frequency of titres and the amount of H.I. antibody are of importance.

The investigations were done on individual sera.

Fig. 8–17 show the experiments employing the human strains of influenza virus A-WS (1933 England), A-PR8 (1934 U.S.A.), A1 (1949 Nederland), and A1 (1956 Nederland).

The first peak in the percentage frequency of H.I. titres against human strains generally coincides with high titres. For the strain A-WS this peak appears around the age of 18, for the strain A-PR8 around the age of 20, and for the strain A1-1949 around the age of 11. Employing the

strain A1 (1956) as antigen, the first peak incidence of titres appears around the age of 11, but in the 3—10 age groups individual sera show a rather high titre.

In old age there is a definite decline in the amount of H.I. antibody in individual sera for the human strains employed (not examined for the strain A-WS) (fig. 18, 19 and 20).

The content of H.I. antibody for swine influenza virus A in individual human sera of different age groups. Fig. 21 shows the percentage frequency of H.I. titres for the swine virus and for the strains of human influenza virus A-PR8 (1934 U.S.A.) and A1 (1949 Nederland) in the age groups 0—42. An incidence of 100% positive titres for the swine virus is reached at the age of 40. The gradual percentage increase of titres against the swine virus in the different age groups is remarkable. The H.I. antibody found in the newborn, must have been caused by maternal antibody.

Fig. 22 shows the percentage frequency and height of the H.I. titres against the swine virus in the age groups 0—100. Although the height of the titres also rises gradually, there is a rather abrupt increase in the height of titres in sera from the age groups 37—40, reaching high values at the age of 40.

Fig. 23 is obtained from a single experiment, using selected sera with the highest H.I. titres per age group. It shows again the rise in the H.I. titres against the swine virus in sera from the age groups 0—44. Fig. 24 shows the same for a few sera collected before the pandemic of A2 influenza. Fig. 25 shows a similar type of experiment, but employing a recently isolated strain of swine influenza virus (A-swine (1957 Wisconsin)). In this experiment there is one particular high titre against this antigen in the age group 10 (repeatedly confirmed), but the age of the donor has not been verified.

Fig. 26 demonstrated that in old age the amount of antibody in individual sera for the swine virus is substantially lower than in the age groups 40—49.

Comparative titrations of individual sera employing the strain of swine influenza virus and human strains. Comparative titrations of individual sera, employing the swine virus and different human strains in the same experiment, demonstrated that especially in older age groups many sera could be found which only showed antibody for the swine virus or showed a titre of antibody for this virus which was much higher than those against the human strains. Experiments done with selected sera collected before the 1957 pandemic, are shown in fig. 27. From the age group 30 on, sera could be found demonstrating H.I. antibody for the swine virus only. In the age group 76 or older, sera showing this antigenic pattern could be regularly found. These titres were generally low.

Fig. 28 shows comparative tirations, performed with pre-pandemic

sera, employing a strain of swine influenza virus of lower avidity ($F_xM_xE_n$) and the human strains A-PR8 and A1-1949. In the age groups 0–29 the numbers of high H.I. titres against the swine virus are lower in proportion than those with high titres against the human strains, but from 40 years onwards the situation is reversed. In the age groups 30–39 the number of high H.I. titres against the swine virus is approximately the same in percentage as that against the human viruses.

Fig. 29 shows comparative titrations with individual sera, selected at random (1958), employing the swine influenza virus and different human influenza virus strains. Except for sera from the newborn, the H.I. antibody content for the swine virus tends to be much lower than that for the human strains before the age of ± 30. In the 36–41 age groups nearly all the titres against the swine virus are higher than those for the human strains.

In fig. 30 the same kind of experiments are shown but here sera in each age group which were selected by preliminary screening tests, did not show H.I. swine antibody. These were examined in the same experiment with other sera from the same age group which showed the highest titres of H.I. antibody for the swine virus. In these experiments it is also clearly seen that the amount of H.I. antibody for the swine virus found over the age of ± 36 rises above that for the human strains.

Mistakes in labelling found by examining the pattern of H.I. antibody for swine virus and human A and A1 strains. In 3 sera errors in the age given on the label of the tubes containing the sera, which had been sent to our laboratory, could be ascertained by the demonstration of an improbable pattern of H.I. antibody for the swine virus and human A and A1 strains with respect to the age indicated. Table 16 shows the data. The age of the persons R, L and C had been labelled respectively 24, 18 and 14 years. In the first serum titrations the pattern I was found which corresponded with that of sera from individuals from 40 years or older. In re-examining the sera obtained by the family physician the pattern II was found, which indicated that the wrong specimens were previously labelled. In exceptional circumstances this procedure might even have importance in forensic medicine.

Absorption experiments. Absorption experiments were done using the swine influenza virus for absorbing H.I. antibody from a few human sera of different age groups (collected in 1961). The sera were tested for their content of H.I. antibody for the swine virus and for different strains of human influenza virus before and after absorption with the swine antigen.

Table 17 shows the results. Although irregularities were seen in some experiments, it can be said that in general the work of Jensen and coworkers (1956) could be confirmed. In the age groups 40 and over heterologous H.I. antibody generally is removed.

DISCUSSION

The explanation of the H.I. antibody pattern for the human A and A1 strains in different age groups. Before an explanation of the observed H.I. antibody patterns in different age groups for strains other than A2 virus can be given it should be noticed that in general they were not substantially altered by the pandemic of A2 influenza in 1957–1958.

Fig. 31 shows the incidence of H.I. antibody for the A2 strain (A2-305 (1957 Japan); egg-ferret-mouse-egg-line) in the age groups 0–42. In 1957–1958 a 4-fold or more anamnestic rise of H.I. titres against the swine virus, due to infection with A2 virus, was found only in 28% (almost exclusively in age groups over 40); against the A-PR8 virus in 8%, and against the A1-1949 virus in 11%. Nearly the same percentage frequencies of rises were seen after vaccination with monovalent A2-vaccine.

The explanation of the H.I. antibody pattern for the human A and A1 strains is not too difficult because the year in which these viruses started to circulate is fairly well known, with the exception of the strain A-WS (1933 England). This strain was the first to be discovered in man (Smith, Andrewes and Laidlaw, 1933).

The strain type A-PR8 (1934 U.S.A.), which was isolated for the first time in Puerto Rico in 1934, caused widespread winter epidemics in both the Northern and Southern hemisphere between 1934 and 1943. In the Netherlands this strain type was isolated in the winter of 1939 and 1941 (in 1937 and 1943 it was not possible to perform any virological investigations in the then prevailing epidemics). The most extensive epidemic caused by this group in this country was in 1941. It is highly probable that the high percentage frequency and the amount of H.I. antibody for this type of influenza virus, present in the sera of the age groups 18–20 and older, reflects the prevalence of the A-PR8 virus in the Netherlands after the birth of persons in these age groups. High titres may have been caused by reinforcement by subsequent infections by strain types of the A1 group. The low H.I. titres against the PR8 virus found in the age groups 10–15, were perhaps induced by the sharing of minor antigen between A1 strains and the PR8 viruses, which possibly was more pronounced in the first strains of the A1 group which appeared.

It should be noticed that Davenport and associates, in 1952, found the first peak titres of H.I. antibody for the PR8 strain type at the age of 17–18, which possibly demonstrated a more extensive spread of this virus in the U.S.A., at an earlier period (1934–1935) than in the Netherlands. We are unable to explain the reason for this difference.

The highest percentage frequency and amount of H.I. antibody for the

strain A1 (1949 Nederland) is found in the ages 9–11. This phenomenon can also be explained in the same way as for the A-PR8 virus and implicates the period of 1947–1949 in which the group of A1 viruses became prevalent.

The explanation of the pattern of the H.I. antibody for the strain type A1-1956, is complicated by the fact that this type is antigenically related to the strain A1-1949. The phenomenon that rather high H.I. titres against the strain A1-1956 can be found in individuals under the age at which a high percentage frequency of titres was found, confirms that this strain type was prevalent shortly before 1958.

The explanation of the pattern of the H.I. antibody for the strain A-WS, relating to the frequency and the height of titres, is impossible since we are uncertain of the time when this strain type first appeared. Possibly absorption tests with sera from different age groups, employing the A-WS strain as the absorbing antigen, may provide a more precise answer to this question. It should be emphasized that the A-WS-strain is antigenically rather strongly related to the A-PR8-strain.

The shift in the age group in which H.I. antibody for strains of influenza virus of high titre first appeared in the period 1935–1958. Before a basis for a discussion of the observed phenomena on the H.I. antibody content in human sera for the swine virus can be stated, it is necessary to examine closely the results of the same kind of experiments done in the period 1935–1937 and described above. This concerns the highly important question as to whether or not a shift in the age group in which H.I. antibody of high titre first appears, can be demonstrated between the two sets of experiments done in the periods 1935–1937 and 1952–1958. Without reasonable doubt this shift in age groups is present in the antibodies for the strains of human influenza virus A-WS and A-PR8.

As the percentage frequency of 1958 sera found to have some antibody for the swine virus is considerable at ages 10–20, the question of the amount of antibody found by earlier workers is of great importance. The difficulty here is that in most earlier experiments mouse protection tests were done and therefore only relative conclusions can be made in comparing the antibody level for the swine virus with that for the human strains A-WS and A-PR8. However, these earlier experiments generally demonstrated that in 1935 the percentage number of positive sera, and thus probably also the height of the titres against the swine virus reached high values between the ages of 10–20 (fig. 1–3). In this respect the experiments of Burnet and Lush using sera collected in 1937 are of great importance, since they were performed with a quantitative virus neutralization test (egg membrane technique). This technique was carefully compared with the mouse protection test and was found to give almost identical results.

Their results are seen in fig. 4. This shows that in 5 newborns, neutral-

izing antibody for the swine virus was present at a higher level than that for the human viruses (A-WS and A-Melbourne (A-PR8-group)). This reflects the antibody content of the sera of the mothers (presumably aged 20–40 years). In the age groups 3–8, swine antibody was absent or much less in amount than that for the human A viruses. In the age groups 10–16 the swine antibody level had risen and equalled that of antibody for the human strains (it was higher in one person aged 12 and in two persons aged 16). At the ages 17–28 and one of 50, comprising 10 individuals, 7 showed a titre of antibody for the swine virus higher than that for the human strains. In other words, Burnet and Lush basically found the same comparative patterns of the antibody titres against the swine influenza virus and the human A strains at the age of 16–17 and over as we have found at the ages of 36–40 and over, making a difference of 20–23 years. This is nearly the same difference in time between the two sets of experiments (1937–1958).

Davenport and associates found the first high H.I. titres against the swine virus in pooled sera collected in 1952 in the age groups 35–36. We found the same phenomenon in individual sera collected in 1958 in the 40 age group. This possibly demonstrates again a shift in age in which the first high titred H.I. antibody for the swine virus was found. The shift almost corresponds exactly with the number of years between the two experiments.

The origin of antibody for swine influenza virus A in man. The main results obtained from this study on the presence of H.I. antibody for the swine virus in man are:

(1) In 1958 in sera from the age groups 1–40, an increase in the percentage frequency and the amount of H.I. antibody for the swine virus was found. In sera from 1952 the same was found in the age groups 1– ± 35 and in sera from 1935–1937 in the age groups 1– ± 20.

(2) In 1958 the titres of swine H.I. antibody increased abruptly from the age group 36 on and often reached high values in the age group 40 and over, which were generally considerable higher than those for human A and A1 strains. It is highly probable, according to determinations with neutralization tests, that in 1937 basically the same phenomenon was present in the age groups around 16–17 years and over.

(3) In sera from ages above 70 years the amount of H.I. antibody for swine virus declined.

(4) In 1958 many sera from persons above the age of 30 (especially in ages above 76) could be found to only show H.I. antibody for the swine virus, generally of a low titre, but none for human A and A1 strains.

(5) From sera of persons in the age groups 40 and over (1961), swine antigen most often absorbs heterologous H.I. antibody for human A and A1 strains.

When we consider all results together, including the corresponding experiments in animals (described above) and the former observations on the epidemiology of swine influenza (see introduction), we agree with the suggestion of Laidlaw and the theory of Shope and Davenport and co-workers. The only reasonable explanation for the observed pattern of swine virus antibody in human beings during the period 1935–1958 is that in the pandemic of 1918 a virus containing the major antigen of the swine influenza virus A started to circulate in man and was the cause of this pandemic. In 1958 the H.I. antibody content of the swine virus in the age groups of 1 to approximately 36 years must have been caused by shared swine virus antigen which was present in later appearing human strains, since it is extremely improbable that strains of swine influenza virus A are still circulating in the community (see also Isaacs, 1957). The high H.I. titres found above the age of ± 36 years most probably were caused by reinforcement through an anamnestic recall which was initiated by influenza virus infections occurring after 1918 by human strains sharing minor swine H.A. antigen with the original strain of the swine type. This assumption is in accordance with the results of the vaccination experiments of Davenport and co-workers described above.

We believe that it is highly probable that the hypothetical human A strains appearing after 1918 and before 1933 were originally derived from the strain of swine influenza virus A. In other words, the new pandemic type of 1918 influenza virus after several years had probably changed its major antigen. This change might have been gradual in time, as we can also postulate as having occurred when the A-PR8 group changed and strains of the A1 group first appeared (e.g. the strain A1-Cam (1946 Australia) was antigenically an intermediate between A and A1 isolates appearing in 1947 (Van der Veen and Mulder, 1950; Mulder and Brans, 1952)). A classic example was the gradual change in antigenic composition of the first isolated strains within the A1 group, and was seen to give rise to the variants A1-1951, A1-1953 and A1-1956, within a period of about 10 years.

The abrupt appearance of the A2 type of strain which caused a new classic pandemic in 1957, and has since been circulating in man, including the year 1962 [1]), has changed the picture completely. In our view a new era of influenza epidemiology had appeared in 1957 caused by a new 'family' of influenza virus A. Although a minor antigenic relationship can be demonstrated to exist between this strain type and the preceding strains, the antigenic 'distance' between A2 and preceding strains is so great, that with the exception of age groups over 70, protective antibody for the A2 virus was nearly absent in the world population of 1957 (Mulder 1957;

[1]) One isolate from 1962 in the Netherlands showed a slight antigenic difference compared with strains from 1957 and 1960.

Mulder and Masurel, 1958; Davoli and Bartolomei Corsi, 1957; Davenport, 1958; Davenport and Hennessy, 1958).

When we consider the alternative explanation for the behaviour of swine antibody in man, as being caused only by the sharing of minor antigen between this type of virus and human strains, we would also have to accept three probable facts: (1) human strains circulating shortly after 1918 would have been more closely related to the swine virus than those circulating after 1933. Otherwise a shift in the age groups in which the first high titred antibody for the swine virus has been found would not have occurred. (2) The hypothetical virus of the 1918 pandemic would have been largely deprived of the swine influenza virus A antigen. Otherwise the occurrence of a classic pandemic of influenza caused by an influenza virus related to the swine influenza virus would not have appeared, since the human race in 1918 would have shown a serum immune pattern with adequate protective antibody for the swine virus before the appearance of the new pandemic strain. (3) The causative virus of 1918 probably would have been circulating only during a short period of time, and would not have been followed by later strains derived from it, since these strains probably were closely related to the swine virus.

If we exclude a 1918 influenza virus B pandemic a tentative conclusion then would be that the 1918 virus possibly was some unknown respiratory virus, or perhaps even the often discussed multiplicity of microbial agents composed of a virus and highly pathogenic respiratory bacterial groups. At present all evidence, from which the histopathology of the 1918 disease is the most conclusive, is against such a hypothesis. The study of fatal cases during the A2 influenza pandemic has clearly shown that the histopathology of the 1918 disease is identical with that of A2 influenza in 1957–1960 (Hers et al., 1958; Hers and Mulder, 1961). This identity is illustrated by the view expressed by Winternitz and co-workers in 1920 that the causative agent of the 1918 disease destroyed the epithelial lining of the respiratory tract and also of the alveoli, thus causing a *'porte d'entrée'* for secondary bacterial invaders in the air-ways and lung tissue.

The antibody content for swine and human virus in sera collected in 1935 and 1936 from people living in St. Helena. In the introduction mention was made of the investigations of Stuart Harris and co-workers on sera collected from people living in St. Helena which had escaped the 1918 pandemic. These investigators had found substantial rises in swine virus antibody during an influenza epidemic on the island in June of 1936. In 1935 the antibody content for swine virus had been found to be absent or very low. It is known that the people in St. Helena did not suffer an outbreak of the disease during the years 1917–1921. Therefore, it is probable that after 1921 the population of St. Helena had been infected with either the original swine vrius, still circulating at that time, or with an antigenic derivative of it which was still closely related to the original

strain of swine influenza virus. In 1936 an anamnestic recall of swine antibody may have occurred which had been initiated by the then prevailing human type of virus A.

Differences in physical properties between the swine influenza virus and human strains. Although the accumulated knowledge on the behaviour of swine antibody in man points clearly to a strain with the major antigen of the swine influenza virus A as the cause of the 1918 pandemic, there is still an incomplete answer to this question. There exist differences in physical properties between the swine influenza virus A and the human A and A1 strains, both in their egg-isolated and in their mouse-lung adapted derivatives. This question is all the more interesting because some of the physical properties of the human A2 strains closely resemble those of the swine influenza virus A. In both, the egg-line strains were found to be non-sensitive to neutralizing inhibitor present in certain animal sera (e.g. mouse, ferret and cattle). Both elute slowly from red cells and in both the haemagglutinin is destroyed before the virus R.D.E. after heating (Rasmussen, 1960; Rasmussen and Hsieh, 1961). All these properties are different from those of human A and A1 isolates.

If we assume that human A strains were originally derived from a human virus possessing the same physical properties as those found in the lines of influenza virus, which have been isolated from swine since 1930, the possibility that these properties have changed during their passage in human tissues must be accepted. Perhaps future experience with the new A2 family may answer the above question.

Are there 'era's' in the epidemiology of influenza A, starting by true pandemics? When we consider the theory that the 1918 pandemic was caused by a strain of 'swine' influenza virus A and when we evaluate this postulate against both the background of the 1957 pandemic of A2 influenza and the general epidemiology of influenza, we are in favour of the old hypothesis of Leichtenstern (1896). This states that influenza (A) is a disease which generally appears in cold season 'conditioned' epidemics which 'follow' and 'belong to' classic (true) pandemics. Before a new pandemic erupts, a rather long period of relative quiescence in the prevalence of the disease may even occur. This was probably the case in the years preceding 1889 and possibly also before 1918. After a true pandemic, seasonal epidemics follow at more or less regular intervals, in which this 'vagabond' virus may change its antigenic composition to be able to continue to circulate in man. In this way even pseudopandemics may occur. The virus progeny in these epidemics retains some of the antigenic component which was present as the major antigen in the pandemic parent strain. If this hypothesis can be proven to be true – and in near future this can be checked – we will be able to define 'era's' of influenza virus A 'families'. Thus we might define the period from

1918–1957 as the 'swine' influenza virus A-era which was then followed by an era of influenza virus A2. There is some evidence that an 'A2' era also may have started in 1889, so that 'pandemic' strains might cause cyclically re-occurring eruptions of true pandemic influenza.

Evidently, in this problem the major question still remains as to where the origin of influenza pandemics lies hidden. This study will be a very important scientific undertaking for future research in influenza (see Rasmussen and Hsieh, 1961).

Through Shope's brilliant work we now know definitely that such a source might be an animal reservoir. In swine from the U.S.A., influenza virus A does not show any major changes in antigenic composition during a long period of habitation. From such a reservoir the virus may perhaps be carried over to the world population when its immunity which was acquired during a former influenza era, has waned.

SUMMARY

In this study the patterns of the percentage frequency and the amount of H.I. antibody for a strain of swine influenza virus A of high avidity for antibody and for strains of human influenza virus A and A1 were investigated in individual human sera from different age groups which were collected in the Netherlands in 1957 and 1958. The results were then compared with the work of Davenport and co-workers (sera from 1952), and also with former experiments done in 1935–1937, employing neutralization tests. Our conclusion is that in 1958, the H.I. antibody for swine influenza virus A above the age of ± 36 years, very probably was originally initiated by a strain of influenza virus containing the major antigen of the swine influenza virus A, isolated by Shope in 1930. This virus abruptly began to circulate in 1918 in man and thus caused the 1918 pandemic of influenza. This view is in accordance with the theory of Shope and the Ann Arbor group of workers (Davenport et al.) and was first suggested by Laidlaw in 1935.

It is suggested that from 1918–1957 an 'era' of the 'swine' influenza virus A 'family' may have prevailed in man, in which periodically antigenically different strains successively emerged from each other. The human subgroups of influenza virus A (1933–1943) and A1 (1946–1957) are known representatives of this family. An antigenic relationship between the derived strains and the original pandemic parent strain of swine influenza virus A remained present in these strains. This relationship was close enough to be able to give rise to low titred antibody for the 'swine' influenza virus A in younger age groups and also to cause a reinforcement of existing antibody originally acquired by people exposed to the 1918 pandemic.

It is further suggested that in 1957 a new 'era' of influenza virus A started abruptly by the appearance of the influenza virus A2, which caused a new true pandemic. This new virus was not hampered by protective antibody in the world population, due to its great antigenic distance from the strains belonging to the 'swine-A family'.

This broad view on influenza epidemiology was originally suggested by Leichtenstern as early as 1896 by carefully studying the epidemiology of the disease.

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FIGURES

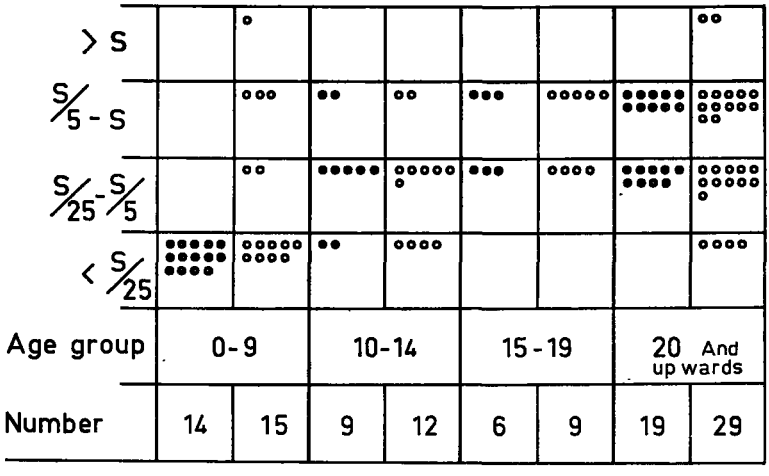

Figure 1. Content of neutralizing antibody in human sera (1935 England) for the strain of swine influenza virus A (black circles) and the human strain of influenza virus A-WS (1933 England) (white circles), using mouse protection tests. In each experiment a standard horse antiserum against the swine and human strain (S) was employed as a reference (composed from data published by Andrewes et al., 1935).

Figure 2

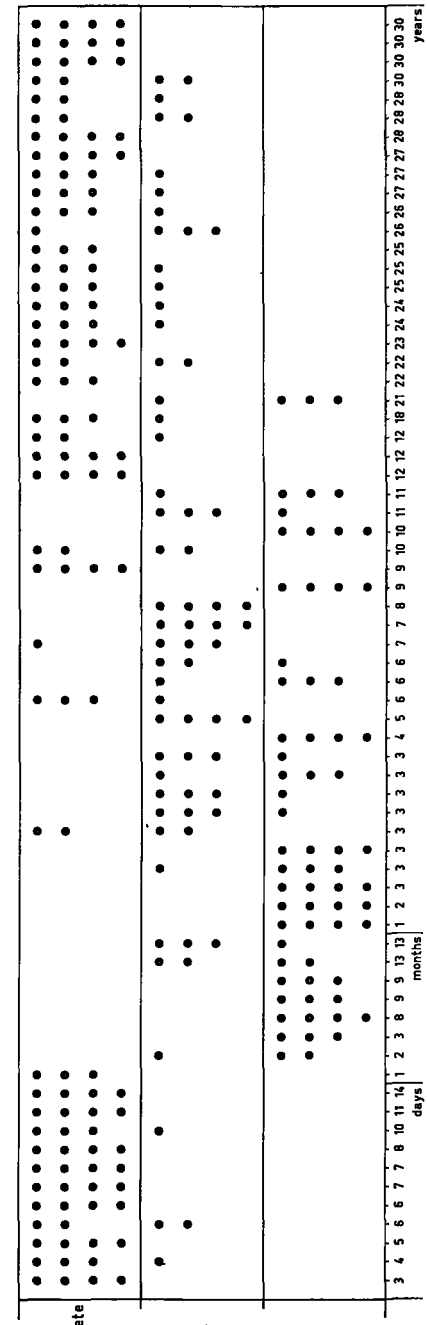

Figure 2. Content of neutralizing antibody in human sera (1935 U.S.A.) for the strain of swine influenza virus A, using mouse protection tests. Each black circle represents an individual mouse (composed from data published by Shope, 1936).

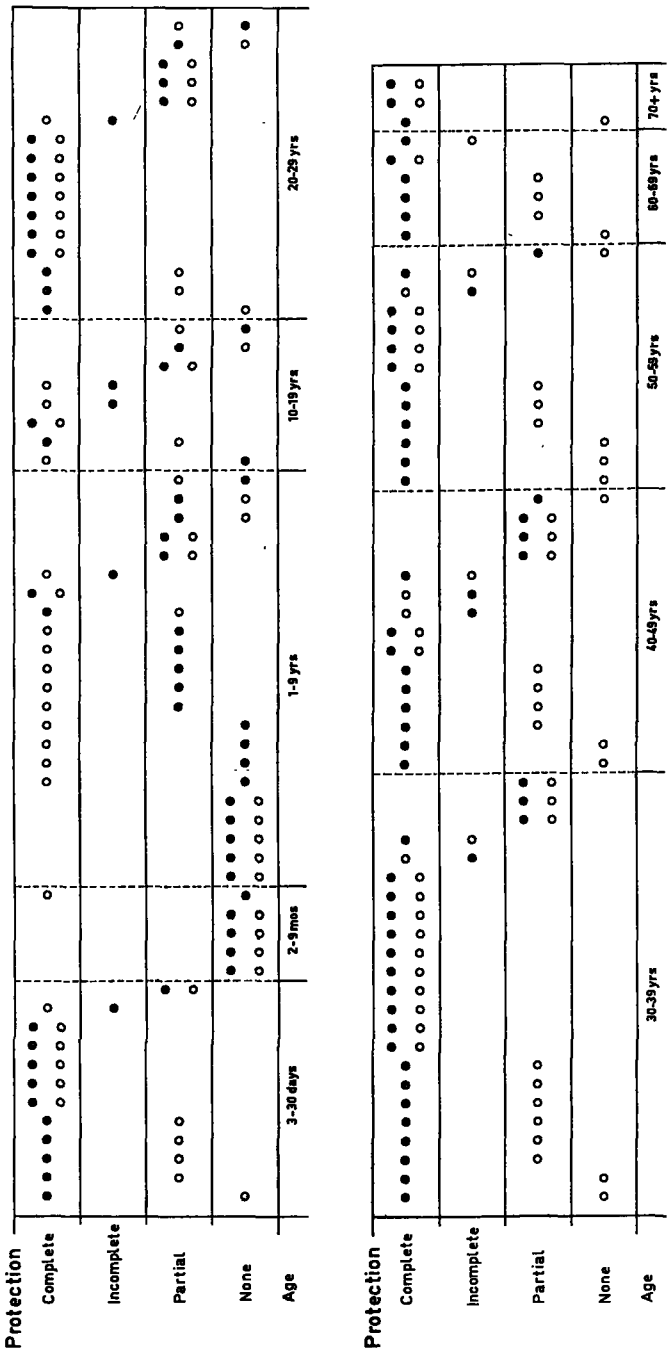

Figure 3. Content of neutralizing antibody in individual human sera (1935 U.S.A.) for both the strain of swine influenza virus A (black circles) and the human strain of influenza virus A-PR8 (1934 U.S.A.) (white circles), using mouse protection tests. The experiments employing the swine virus were done by Shope, and those employing the human virus by Francis and Magill (composed from data published by Shope, 1936, and Francis and Magill, 1936).

Figure 4

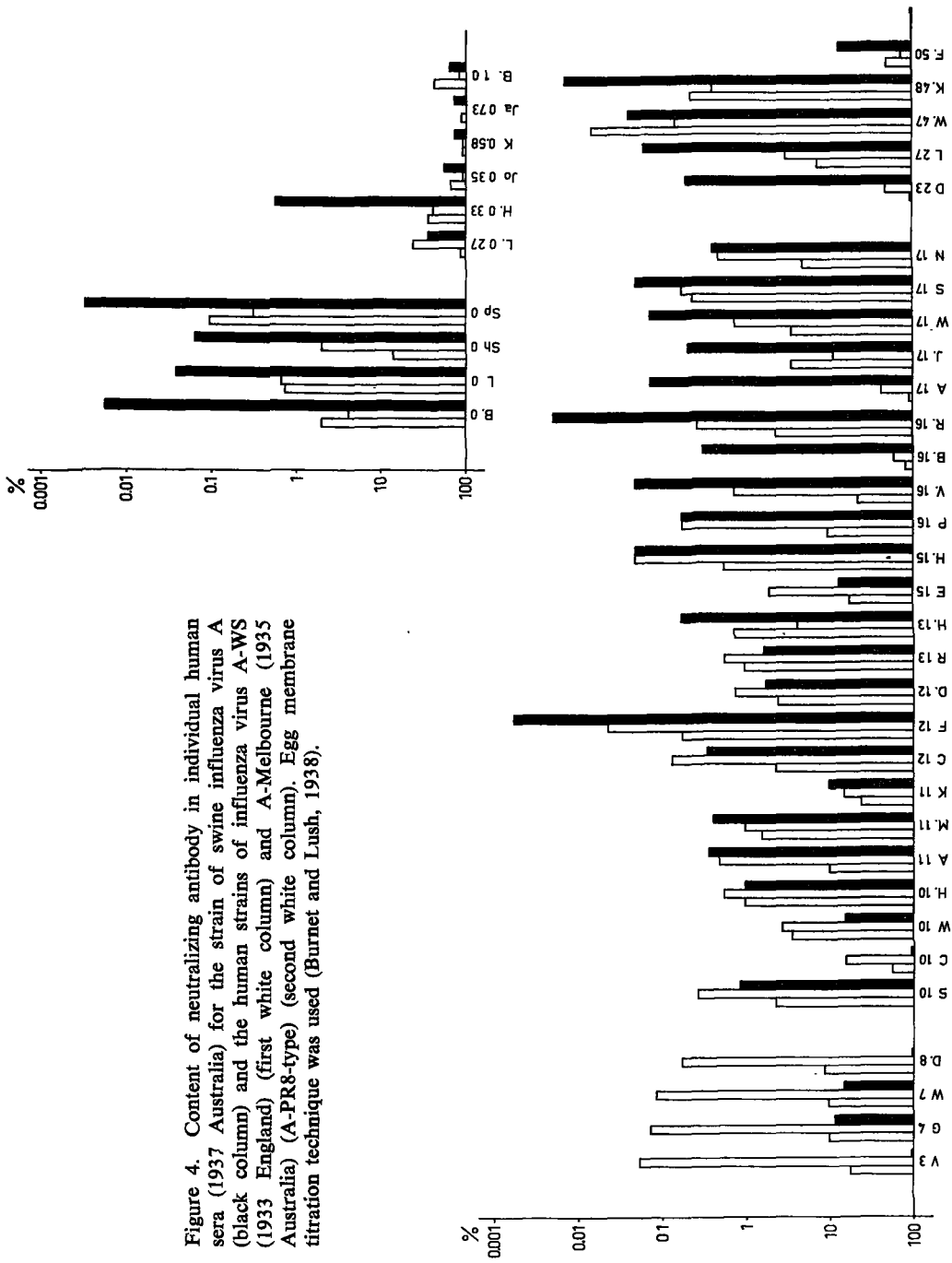

Figure 4. Content of neutralizing antibody in individual human sera (1937 Australia) for the strain of swine influenza virus A (black column) and the human strains of influenza virus A-WS (1933 England) (first white column) and A-Melbourne (1935 Australia) (A-PR8-type) (second white column). Egg membrane titration technique was used (Burnet and Lush, 1938).

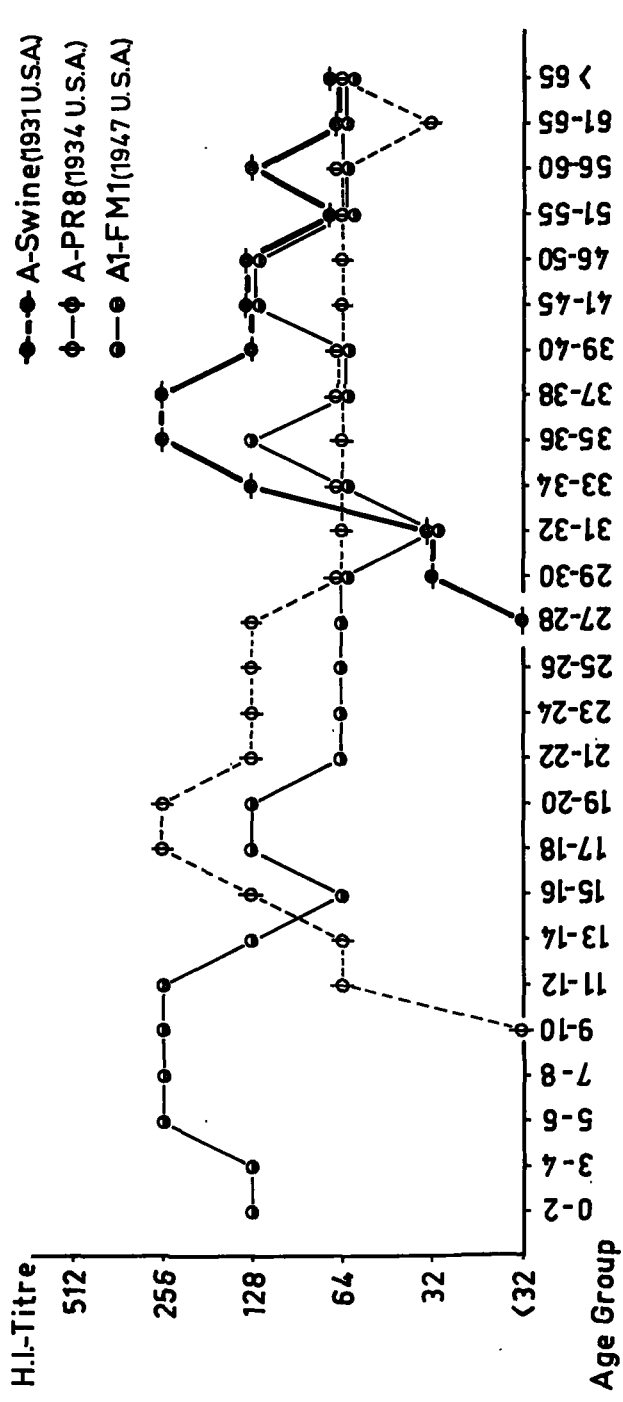

Figure 5. Content of neutralizing antibody in pools of human sera (1952 U.S.A.) for the strain of swine influenza virus A (1931 U.S.A.) and the human strains of influenza virus A-PR8 (1934 U.S.A.) and A1-FM1 (1947 U.S.A.), using the H.I. test (composed from data published by Davenport et al., 1953).

Figure 6

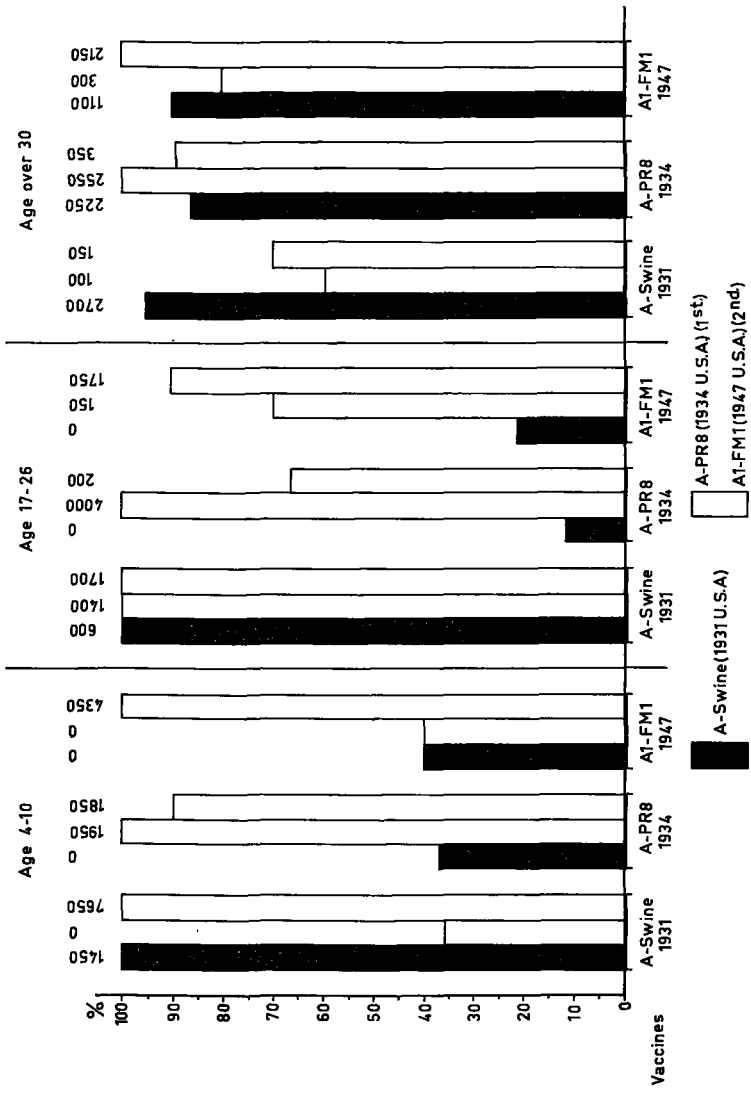

Figure 6. H.I. antibody response in 3 age-groups to monovalent influenza virus vaccine (Swine A, A-PR8 and A1-FM1). (Only the percentage of positive responses occurring after vaccination are shown). The difference in the amount of antibody between the mean geometric titres of the pre- and post-vaccination sera is shown above each column (composed from data published by Hennessy and Davenport, 1958).

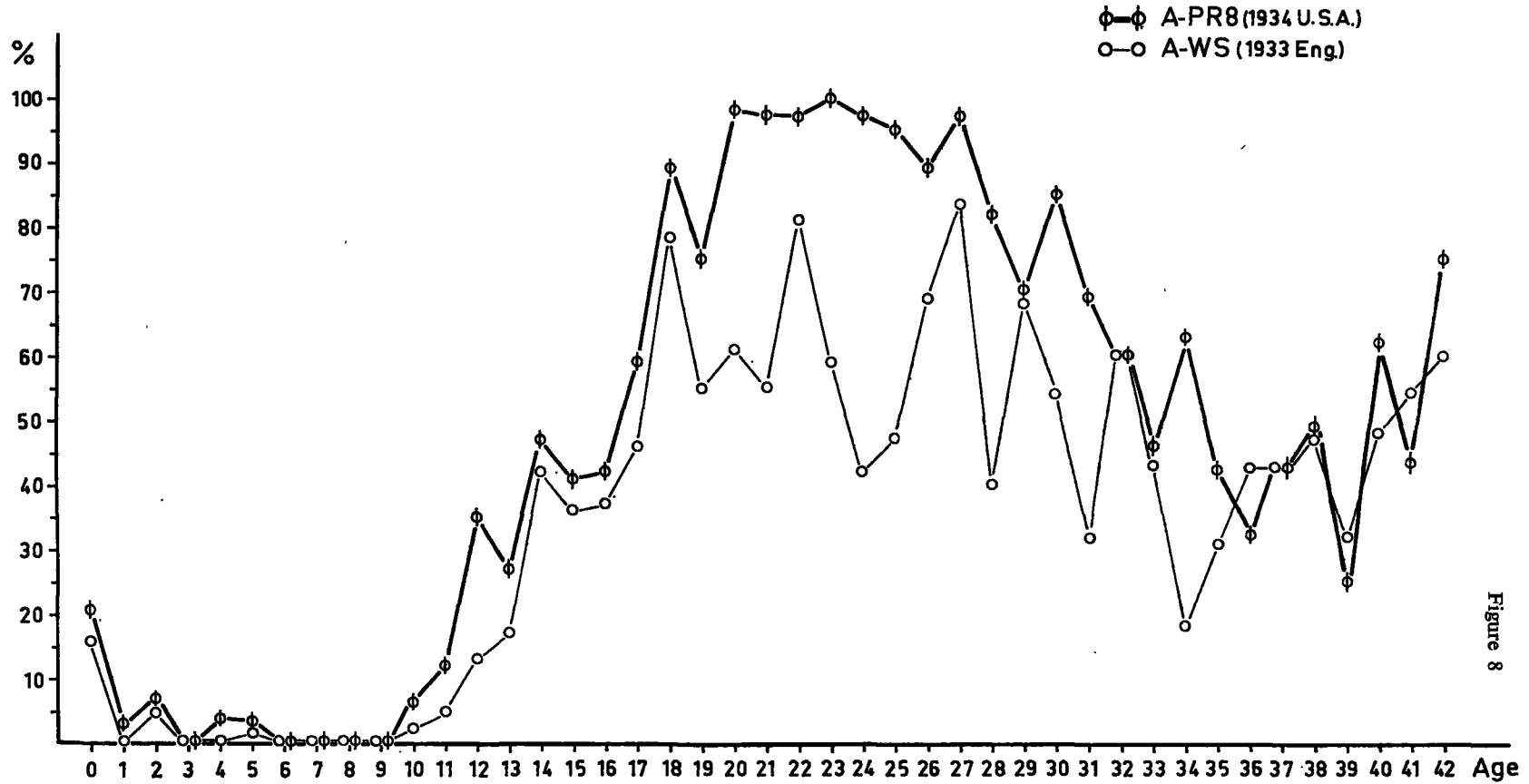

Figure 8

Figure 8. Percentage frequency of H.I. antibody for the human strains of influenza virus A-WS (1933 England) and A-PR8 (1934 U.S.A.) in the age groups 0—42 (sera from 1958). The strains employed in this and all following experiments were adapted to mice.

Figure 9

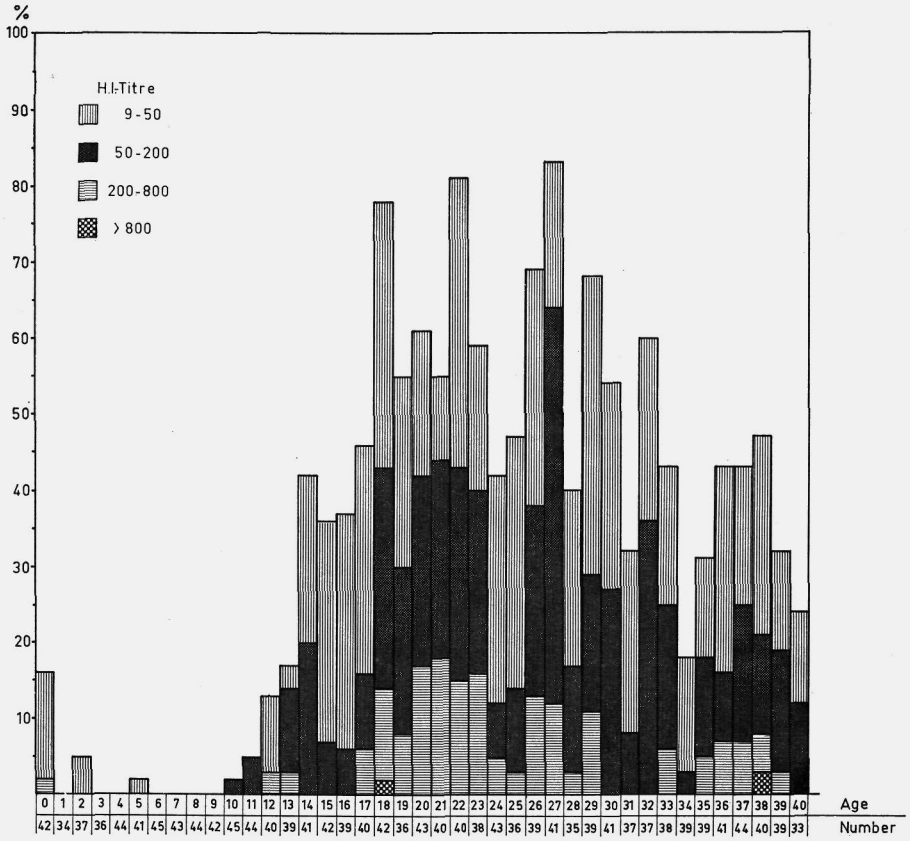

Figure 9. Height of H.I. antibody for the strain of influenza virus A-WS (1933 England) in individual sera of the age groups 0—40 (sera from 1958).

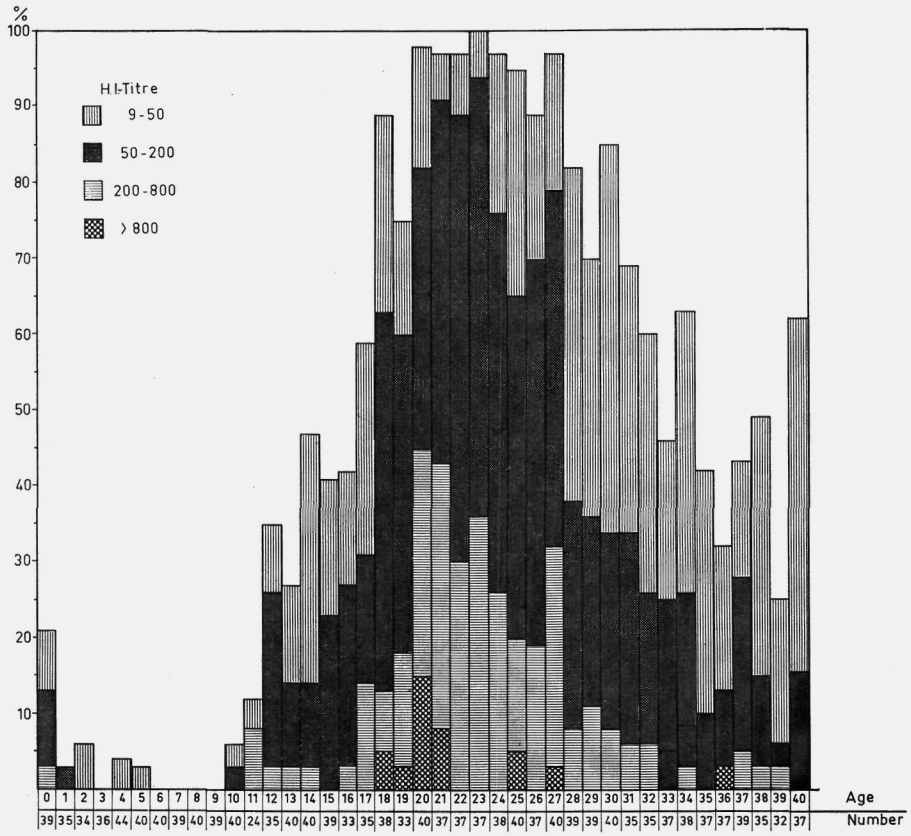

Figure 10. Height of H.I. antibody for the strain of influenza virus A-PR8 (1934 U.S.A.) in individual sera of the age groups 0—40 (sera from 1958).

Figure 11

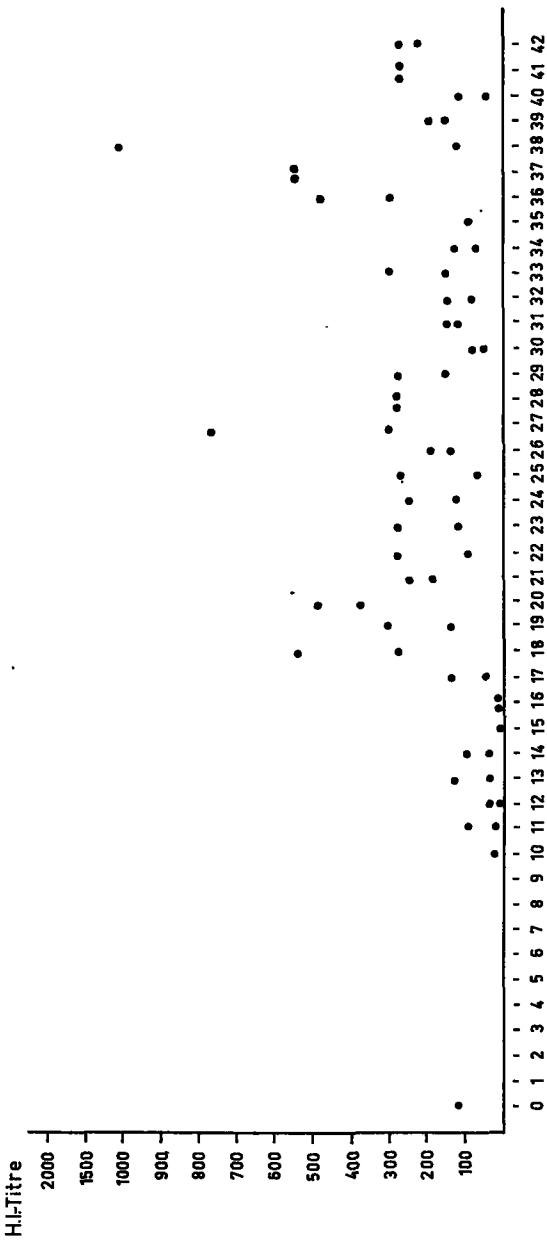

Figure 11. H.I. antibody for the strain of influenza virus A-WS (1933 England) in sera from 1958, selected from preceding experiments, having the highest titre of H.I. antibody in the age groups 0—42 (1 experiment).

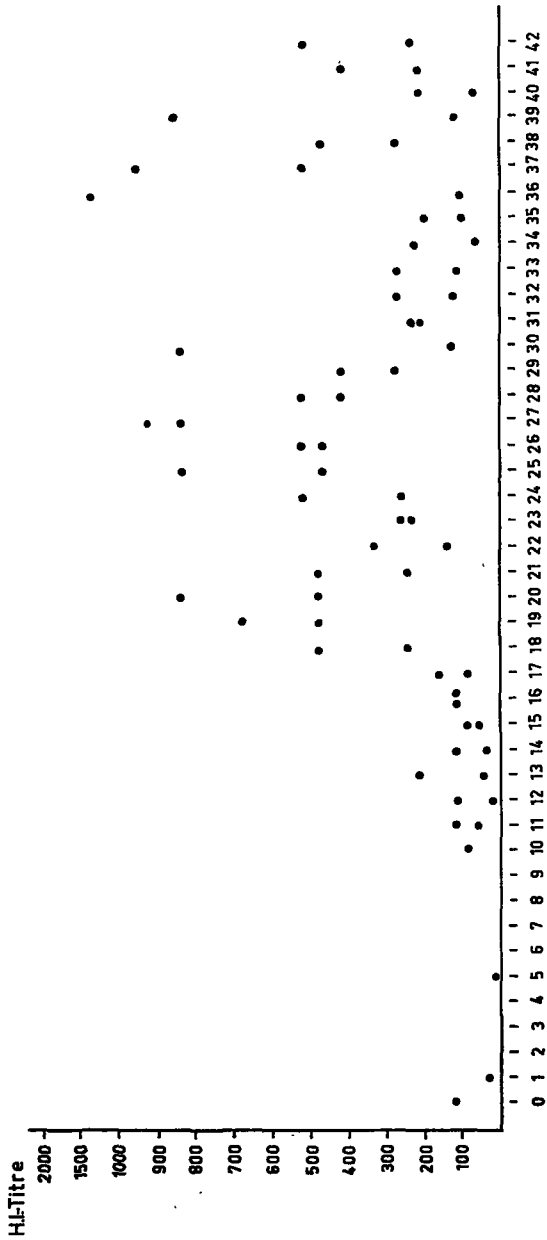

Figure 12. H.I. antibody for the strain of influenza virus A-PR8 (1934 U.S.A.) in sera from 1958, selected from preceding experiments, having the highest titre of H.I. antibody in the age groups 0—42 (1 experiment).

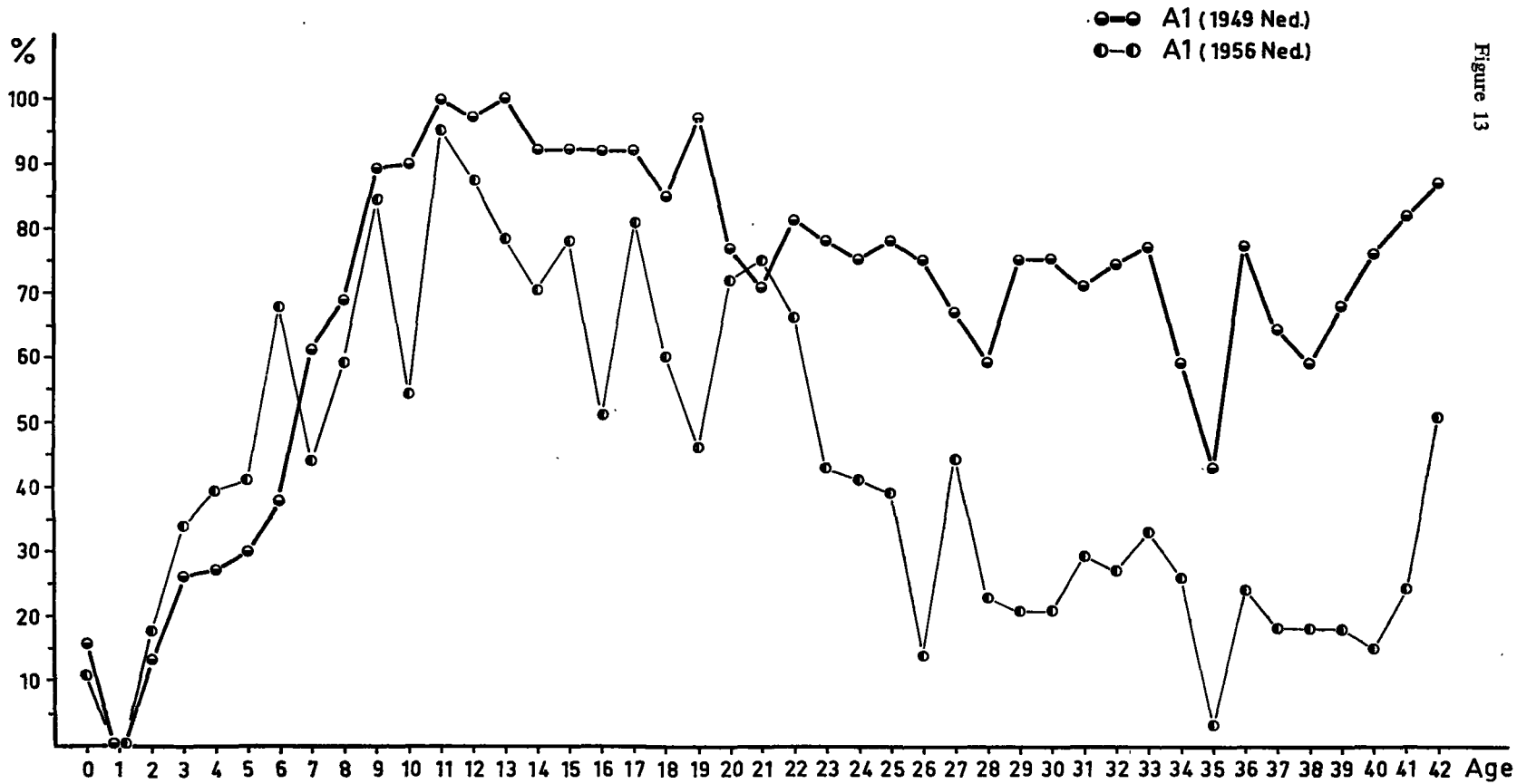

Figure 13

Figure 13. Percentage frequency of H.I. antibody for the human strains of influenza virus A1 (1949 Nederland) and A1 (1956 Nederland) in the age groups 0—42 (sera from 1958).

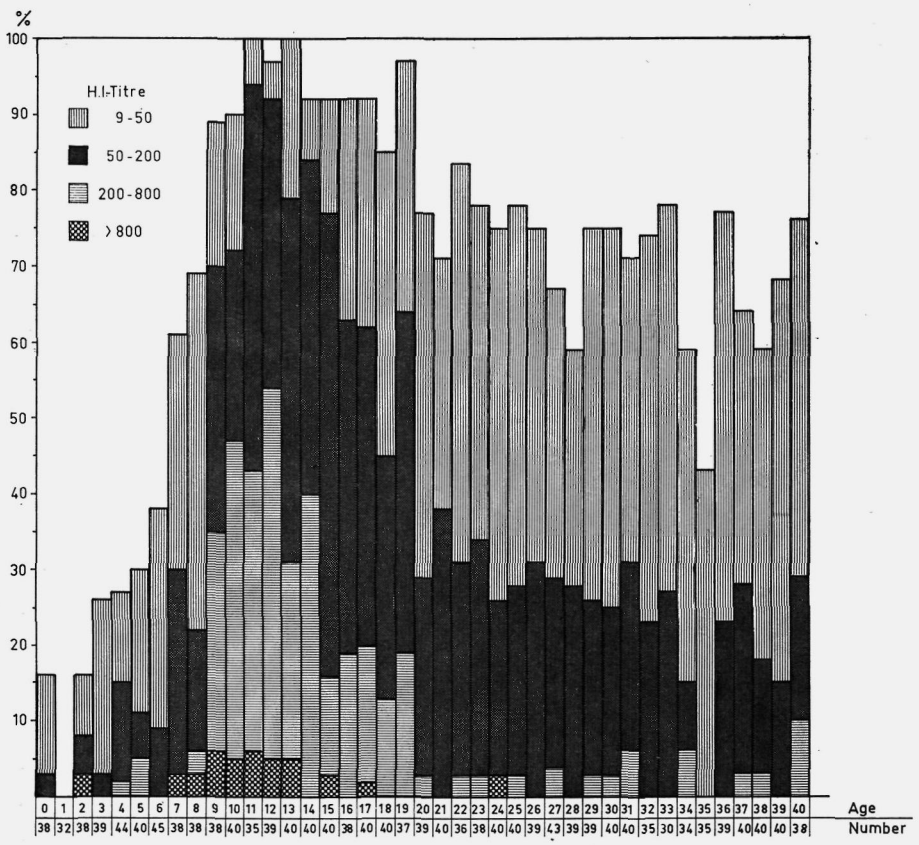

Figure 14. Height of H.I. antibody for the strain of influenza virus A1 (1949 Nederland) in individual sera of the age groups 0—40 (sera from 1958).

Figure 15

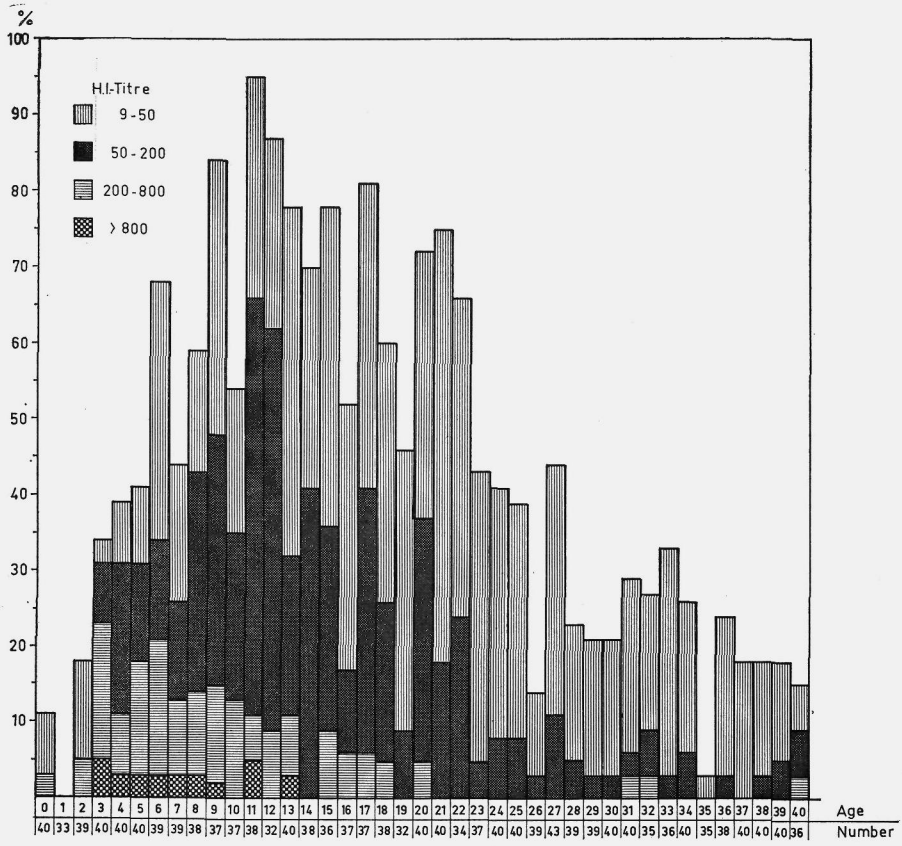

Figure 15. Height of H.I. antibody for the strain of influenza virus A1 (1956 Nederland) in individual sera of the age groups 0—40 (sera from 1958).

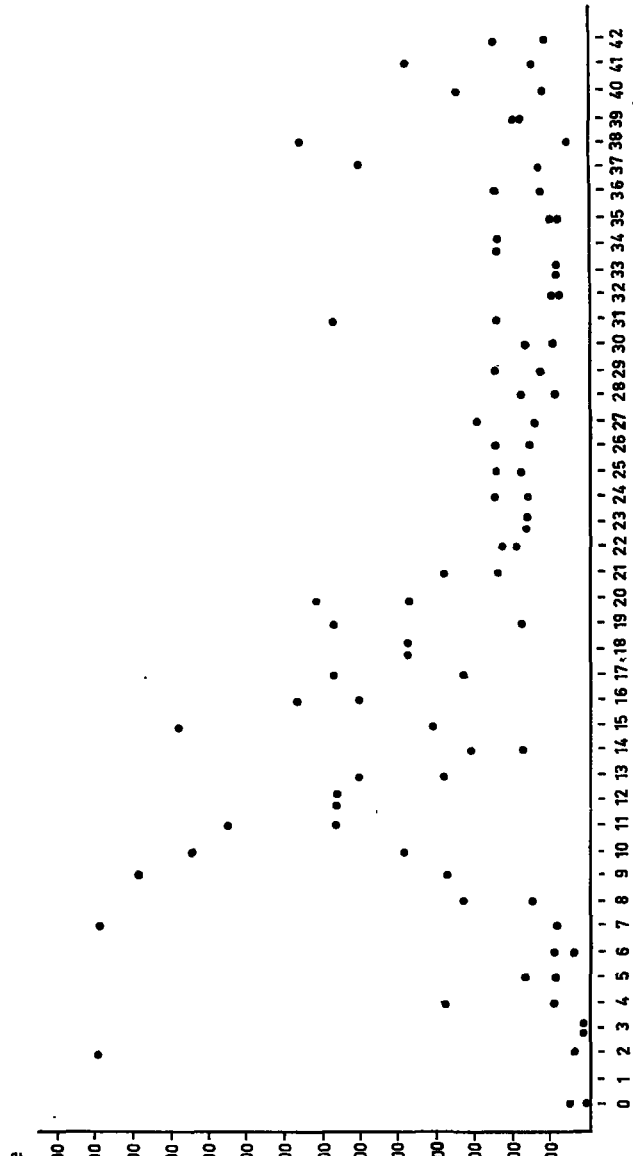

Figure 16. H.I. antibody for the strain of influenza virus A1 (1949 Nederland) in sera from 1958, selected from preceding experiments, having the highest titre of H.I. antibody in the age groups 0—42 (1 experiment).

Figure 17

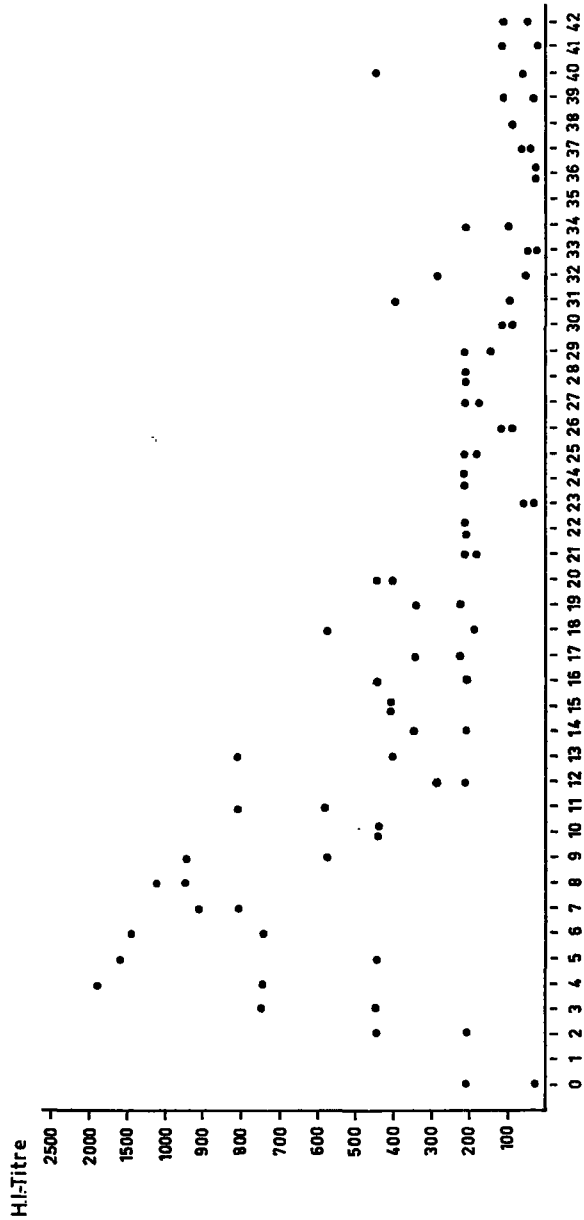

Figure 17. H.I. antibody for the strain of influenza virus A1 (1956 Nederland) in sera from 1958, selected from preceding experiments, having the highest titre of H.I. antibody in the age groups 0—42 (1 experiment).

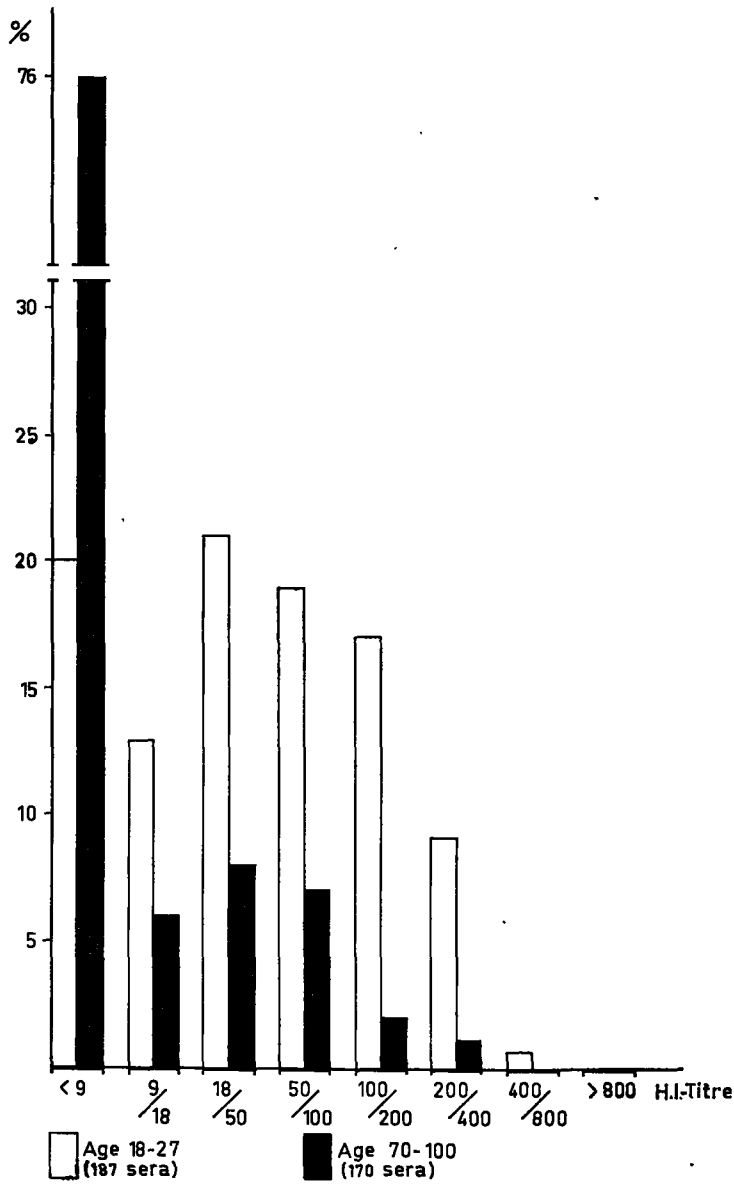

Figure 18. Comparative titrations in human sera, from the 18—27 and 70—100 age groups (1958), employing the strain of influenza virus A-PR8 (1934 U.S.A.). Approximately the same number of sera from both age groups were titrated in the same experiment (6 experiments).

Figure 19

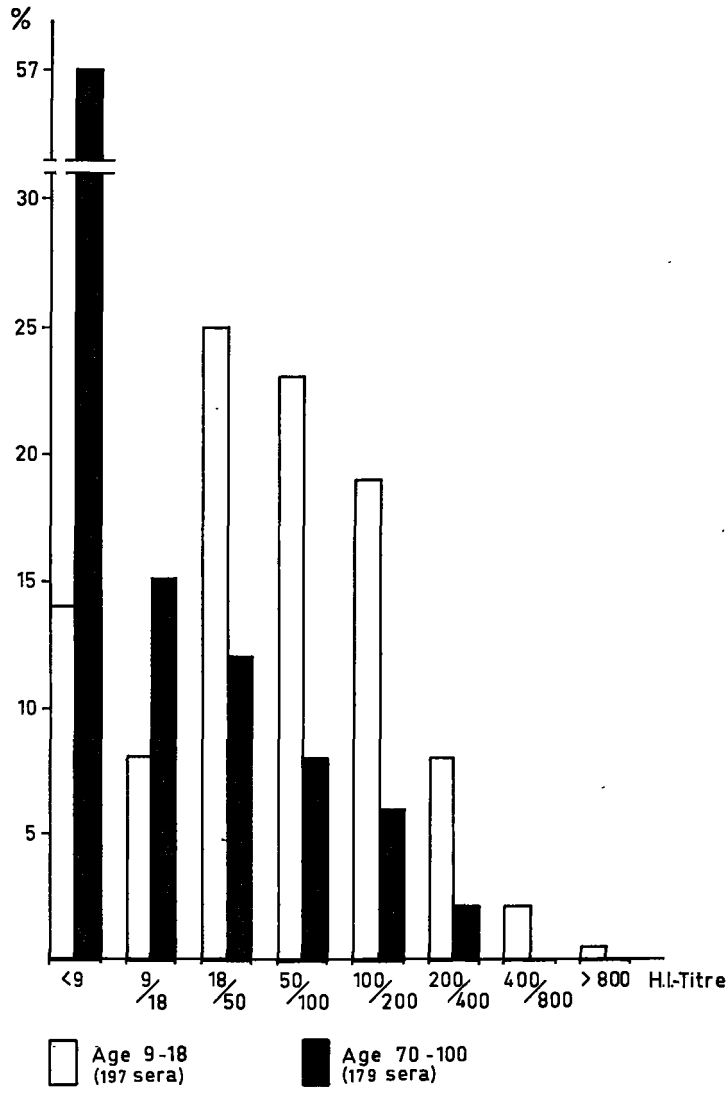

Figure 19. Comparative titrations in human sera, from the 9—18 and 70—100 age groups (1958), employing the strain of influenza virus A1 (1949 Nederland). Approximately the same number of sera from both age groups were titrated in the same experiment (6 experiments).

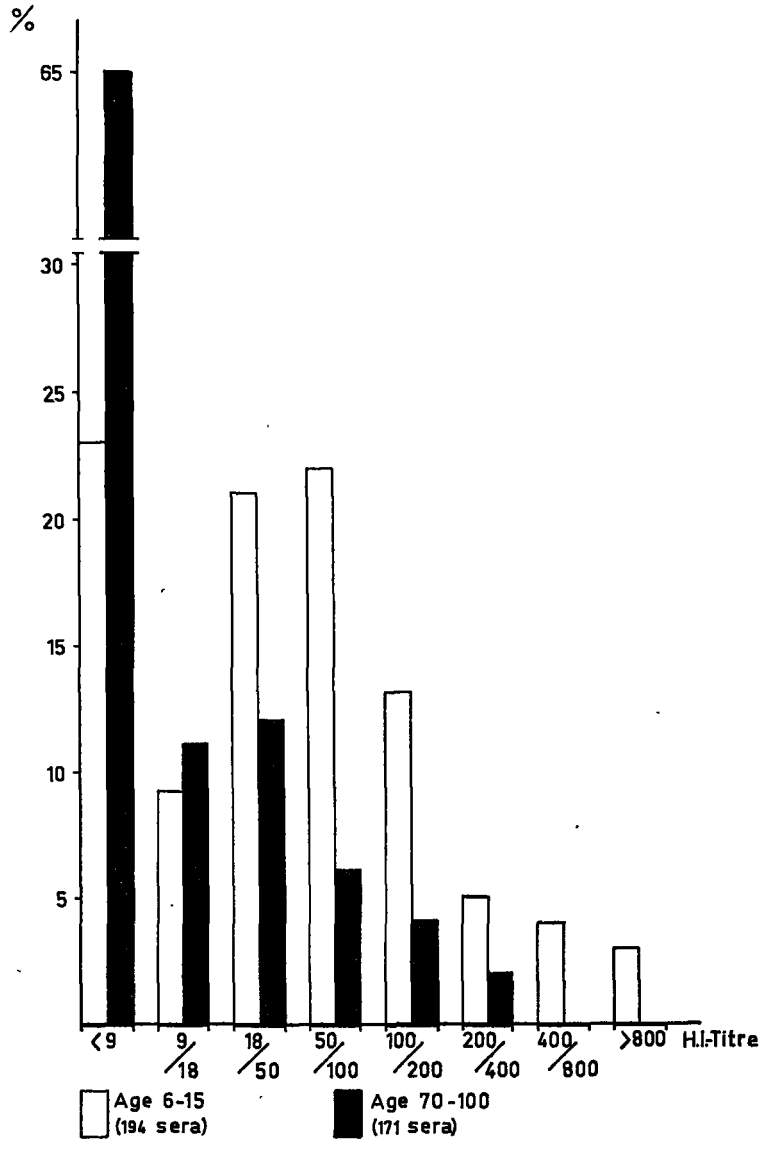

Figure 20. Comparative titrations in human sera, from the 6—15 and 70—100 age groups (1958), employing the strain of influenza virus A1 (1956 Nederland). Approximately the same number of sera from both age groups were titrated in the same experiment (5 experiments).

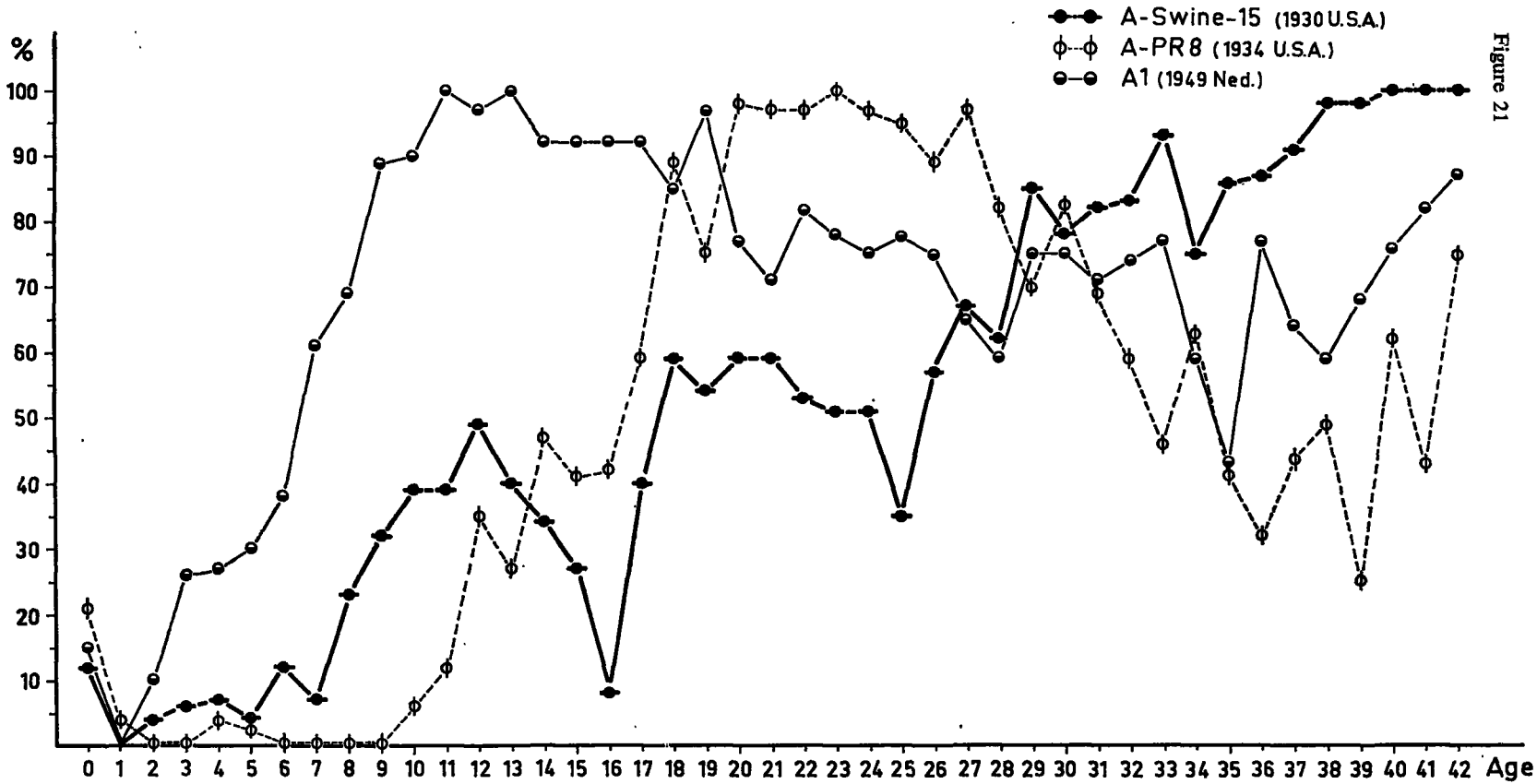

Figure 21

Figure 21. Percentage frequency of H.I. antibody for the strain of swine influenza virus A and for the strains of human influenza virus A-PR8 (1934 U.S.A.) and A1 (1949 Nederland) in the age groups 0—42 (sera from 1958).

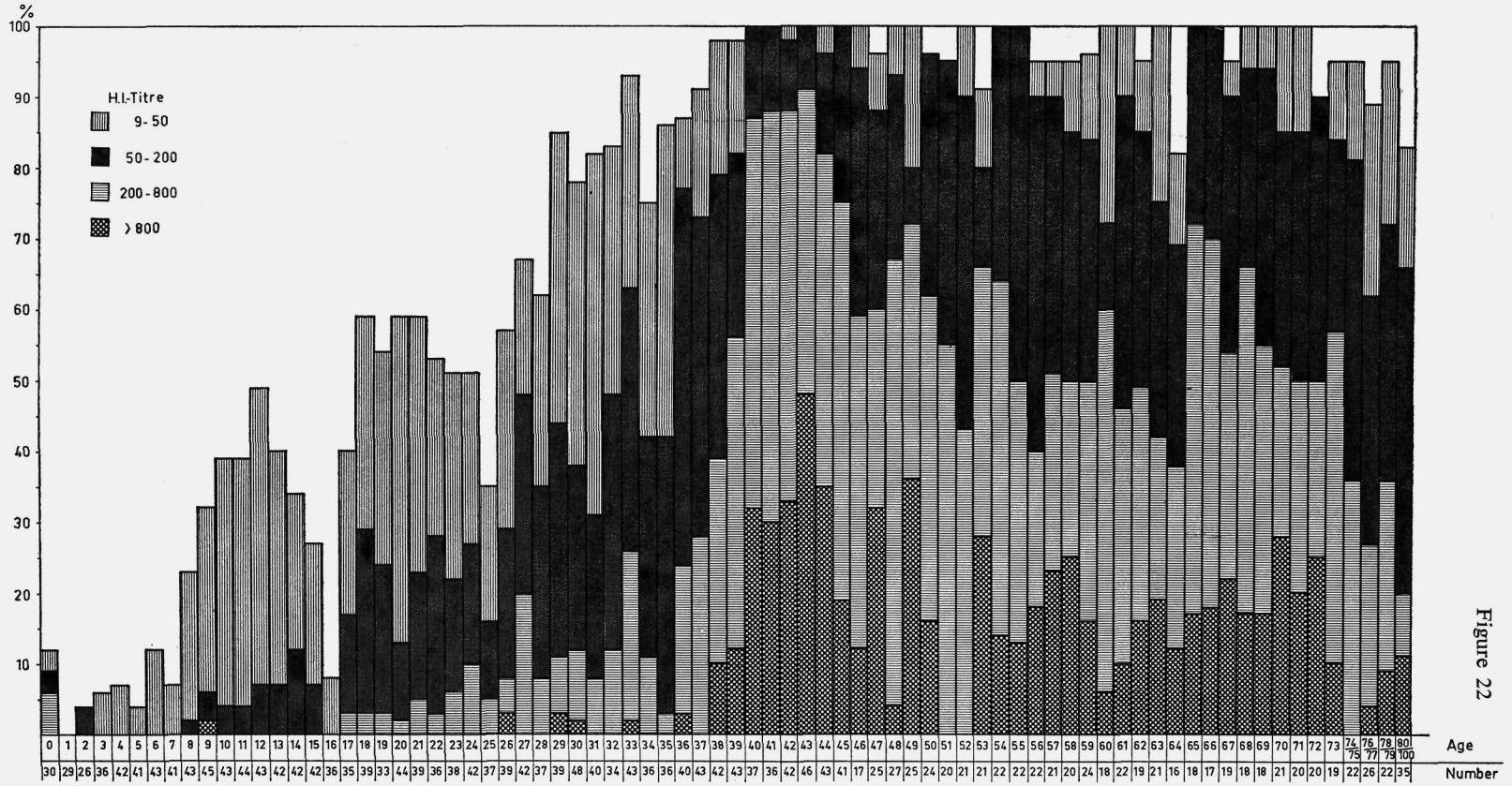

Figure 22

Figure 22. Height of H.I. antibody for the strain of swine influenza virus A in individual sera of the age groups 0—100 (sera from 1958).

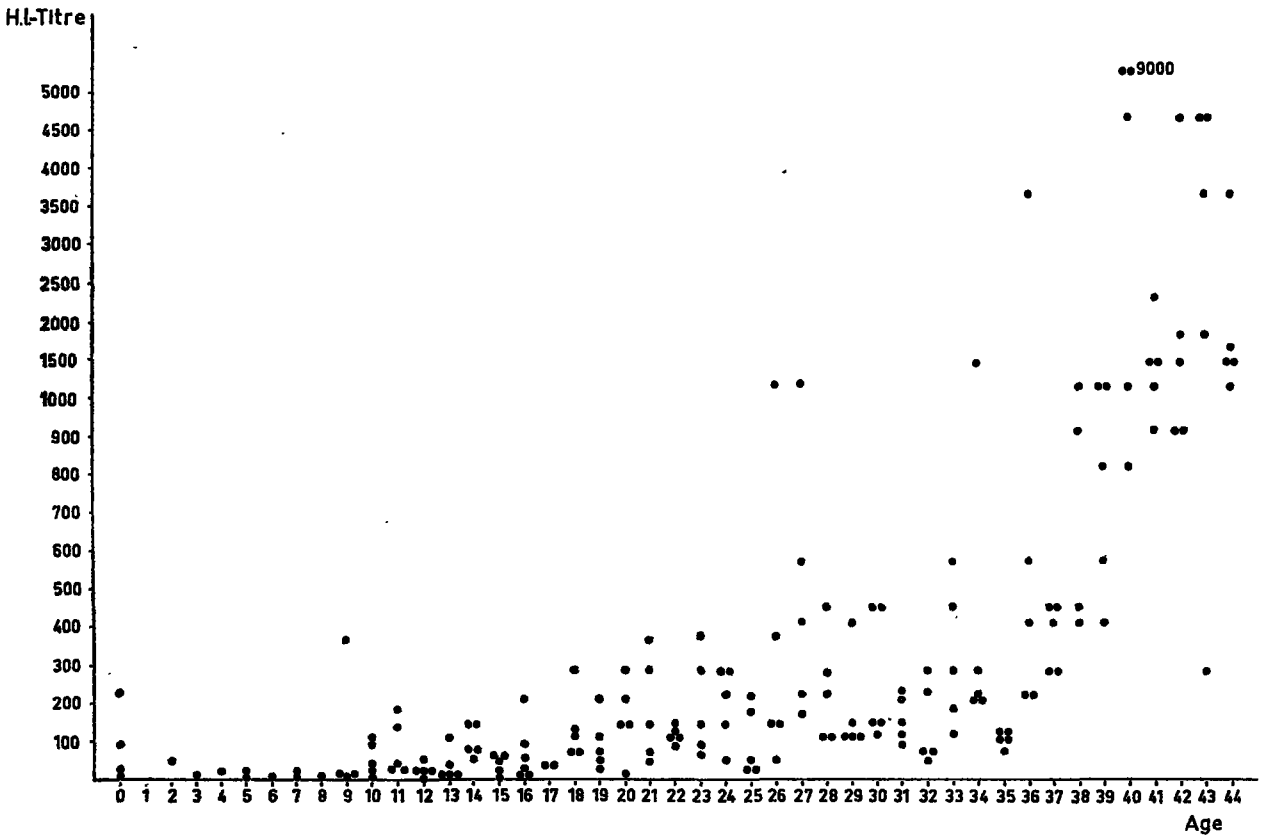

Figure 23. H.I. antibody for the strain of swine influenza virus A in sera from 1958, selected from preceding experiments, having the highest titre of H.I. antibody in the age groups 0—44 (1 experiment).

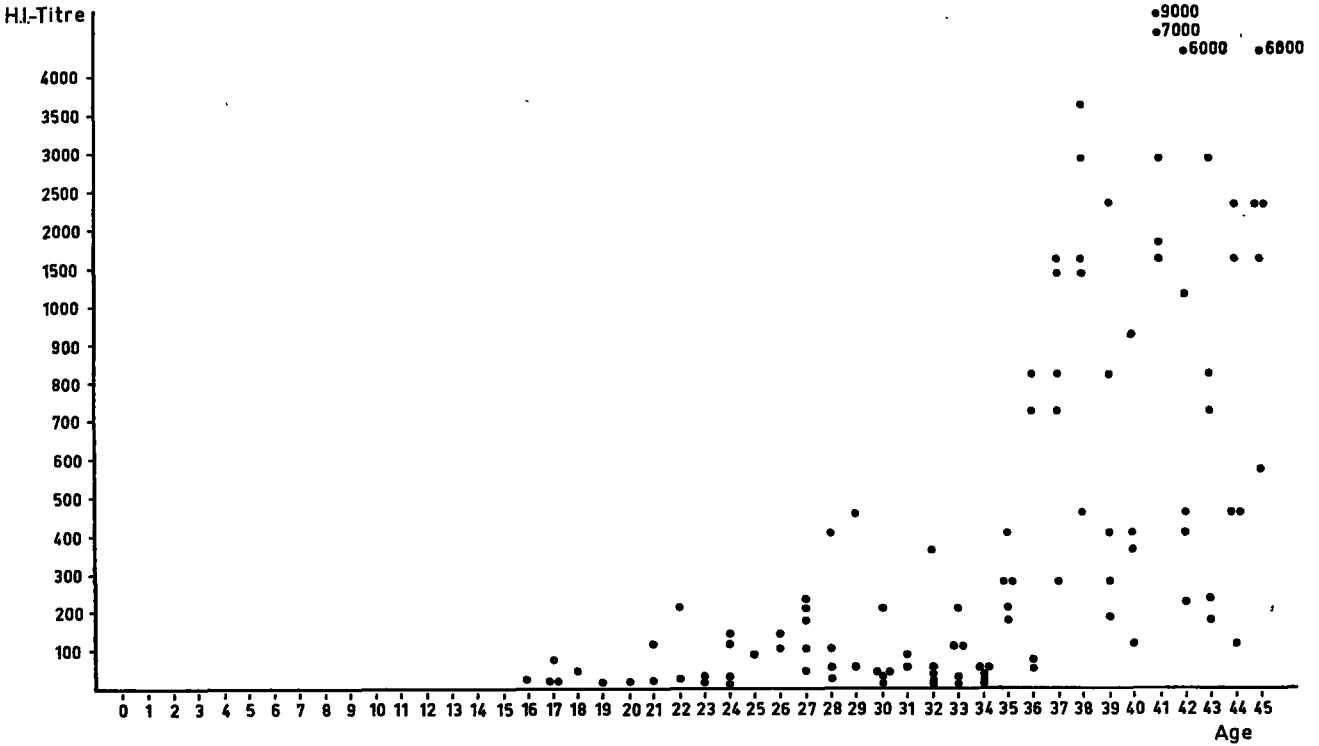

Figure 24. H.I. antibody for the strain of swine influenza virus A in prepandemic sera from 1957, selected from preceding experiments, having the highest titre of H.I. antibody in the age groups 0—45 (1 experiment). Only a few sera were available from the younger age groups.

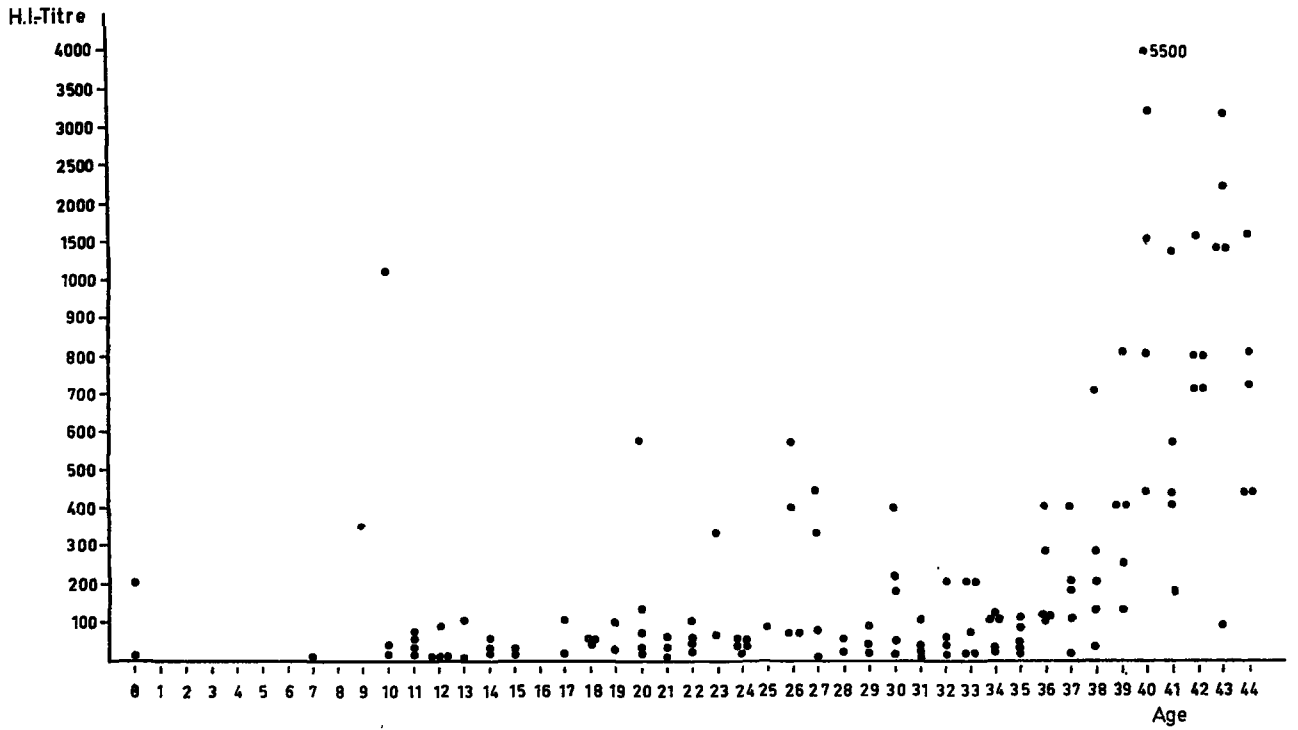

Figure 25. H.I. antibody for a recently isolated strain of swine influenza virus A (1957 Wisconsin) in selected sera from 1958 with the highest titre of H.I. antibody for the strain of swine influenza A (1930 U.S.A.) in the age groups 0—44 (1 experiment).

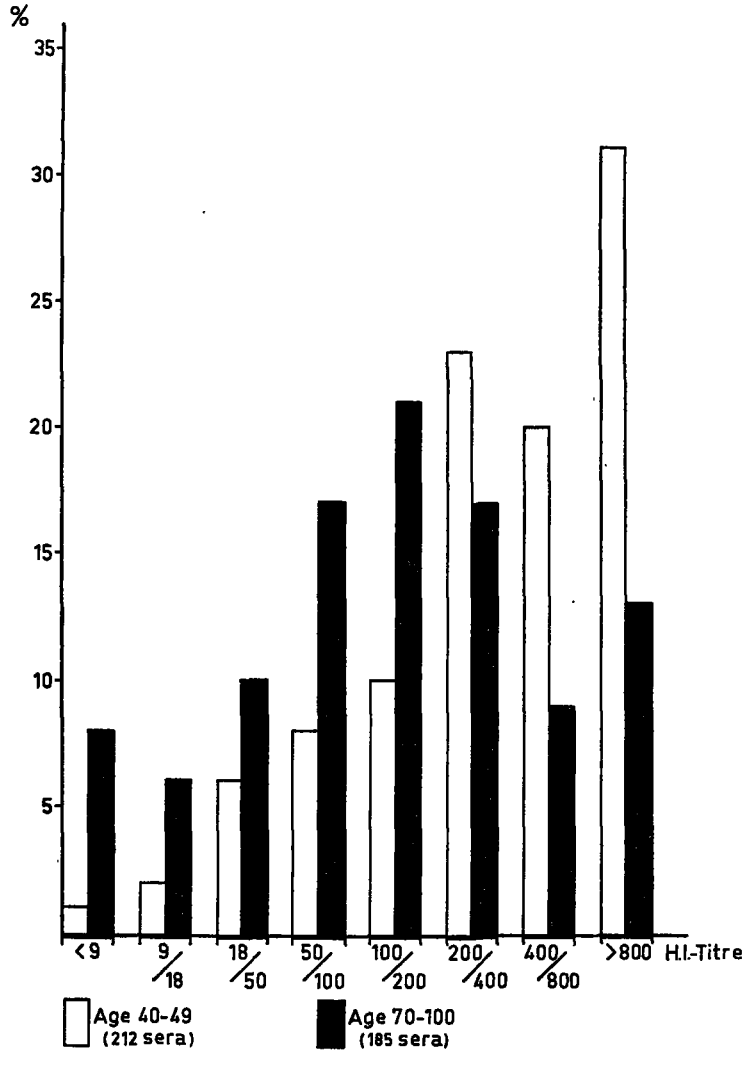

Figure 26. Comparative titrations in human sera, from the 40—49 and 70—100 age groups (1958), employing the strain of swine influenza virus A. Approximately the same number of sera from both age groups were titrated in the same experiment (3 experiments).

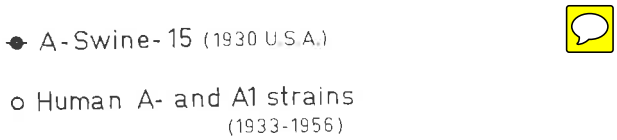

Figure 27. Height of H.I. antibody for the strain of swine influenza virus A and different human strains of influenza virus A and A1 in sera from the age groups 30—90 (prepandemic sera from 1957). The sera were selected from preceding experiments. In 32 persons (marked with an asterisk) only H.I. antibody for the swine virus was present (these experiments were repeated again). The H.I. tests were performed using all strains in the same experiment.

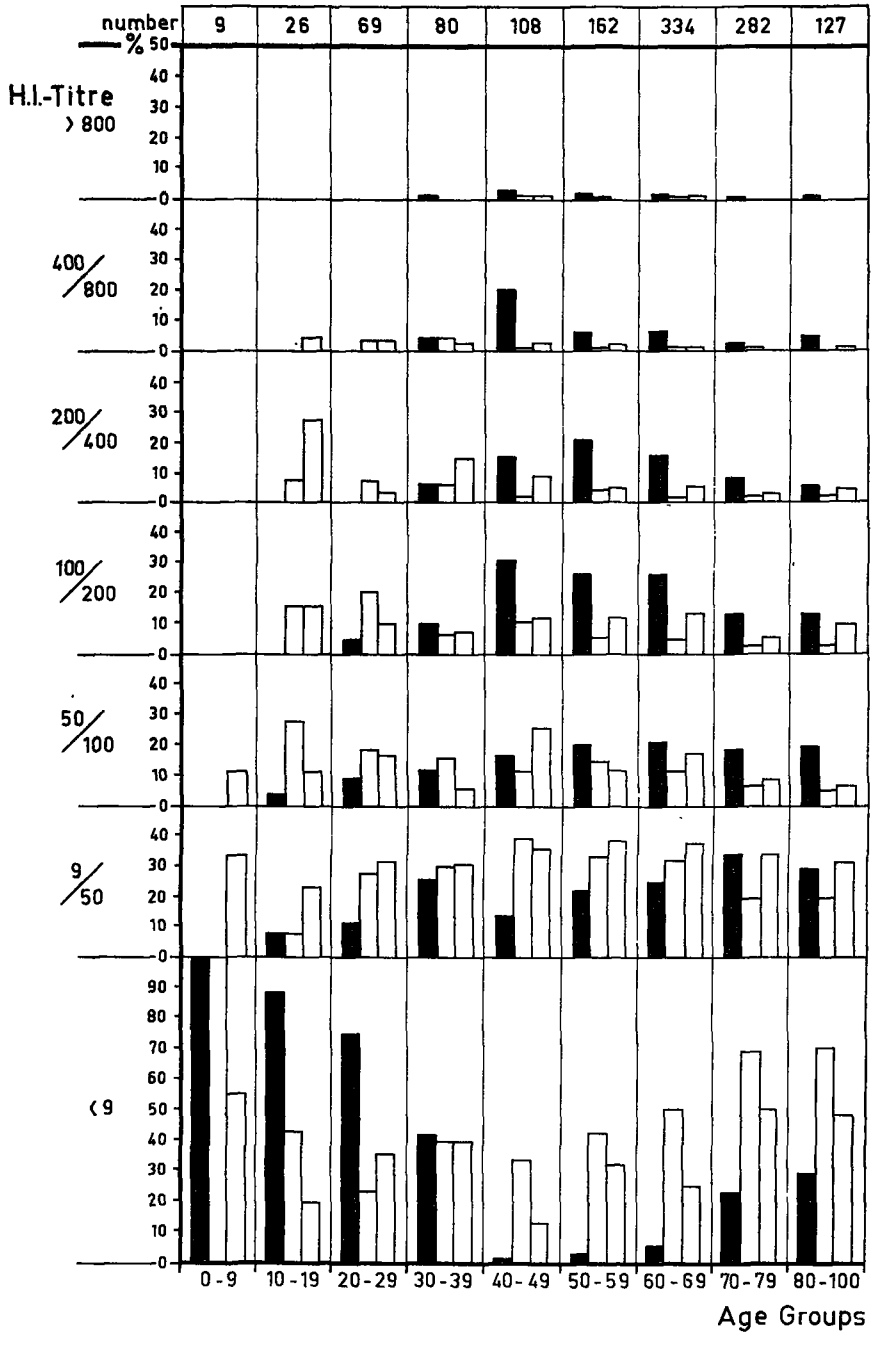

Figure 28. Comparative titres of H.I. antibody for the strain of swine influenza virus A (F_xM_xE; low avidity strain) (black column) and the human strains of influenza virus A-PR8 (1934 U.S.A.) (first white column) and A1 (1949 Nederland) (second white column) in pre-pandemic sera from 1957. Titrations were performed employing all 3 strains of virus in the same experiment.

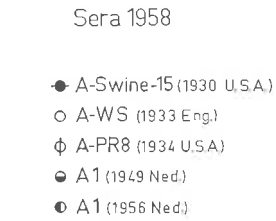

Figure 29. Height of H.I. antibody for the strain of swine influenza virus A and different human strains of influenza virus A and A1 found in sera of the age groups 0—42. The sera were selected at random (sera from 1958). The H.I. tests, marked with an asterisk, were performed using all strains in the same experiments. Therefore, the results of this whole experiment is only of relative importance.

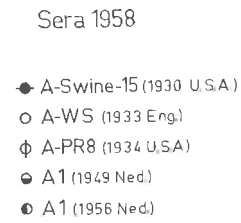

Figure 29 (continued). Height of H.I. antibody for the strain of swine influenza virus A and different human strains of influenza virus A and A1 found in sera of the age groups 0—42. The sera were selected at random (sera from 1958). The H.I. tests, marked with an asterisk, were performed using all strains in the same experiments. Therefore, the results of this whole experiment is only of relative importance.

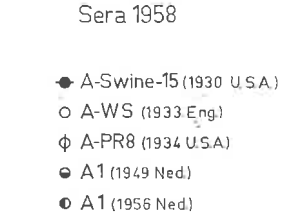

Figure 29 (continued). Height of H.I. antibody for the strain of swine influenza virus A and different human strains of influenza virus A and A1 found in sera of the age groups 0—42. The sera were selected at random (sera from 1958). The H.I. tests, marked with an asterisk, were performed using all strains in the same experiments. Therefore, the results of this whole experiment is only of relative importance.

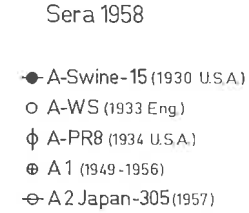

Figure 30. Height of H.I. antibody for the strain of swine influenza virus A and different human strains of influenza virus A, A1 and A2 in sera of the age groups 0—41. From preceding experiments, sera were selected per age group showing no H.I. antibody for the swine virus, and other sera showing the highest titre of H.I. antibody for this virus. The H.I. tests were performed using all strains in the same experiment.

Figure 30 (continued). Height of H.I. antibody for the strain of swine influenza virus A and different human strains of influenza virus A, A1 and A2 in sera of the age groups 0—41. From preceding experiments, sera were selected per age group showing no H.I. antibody for the swine virus, and other sera showing the highest titre of H.I. antibody for this virus. The H.I. tests were performed using all strains in the same experiment.

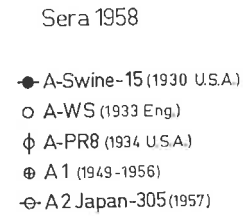

Figure 30 (continued). Height of H.I. antibody for the strain of swine influenza virus A and different human strains of influenza virus A, A1 and A2 in sera of the age groups 0—41. From preceding experiments, sera were selected per age group showing no H.I. antibody for the swine virus, and other sera showing the highest titre of H.I. antibody for this virus. The H.I. tests were performed using all strains in the same experiment.

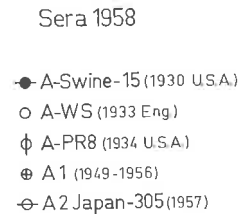

Figure 30 (continued). Height of H.I. antibody for the strain of swine influenza virus A and different human strains of influenza virus A, A1 and A2 in sera of the age groups 0—41. From preceding experiments, sera were selected per age group showing no H.I. antibody for the swine virus, and other sera showing the highest titre of H.I. antibody for this virus. The H.I. tests were performed using all strains in the same experiment.

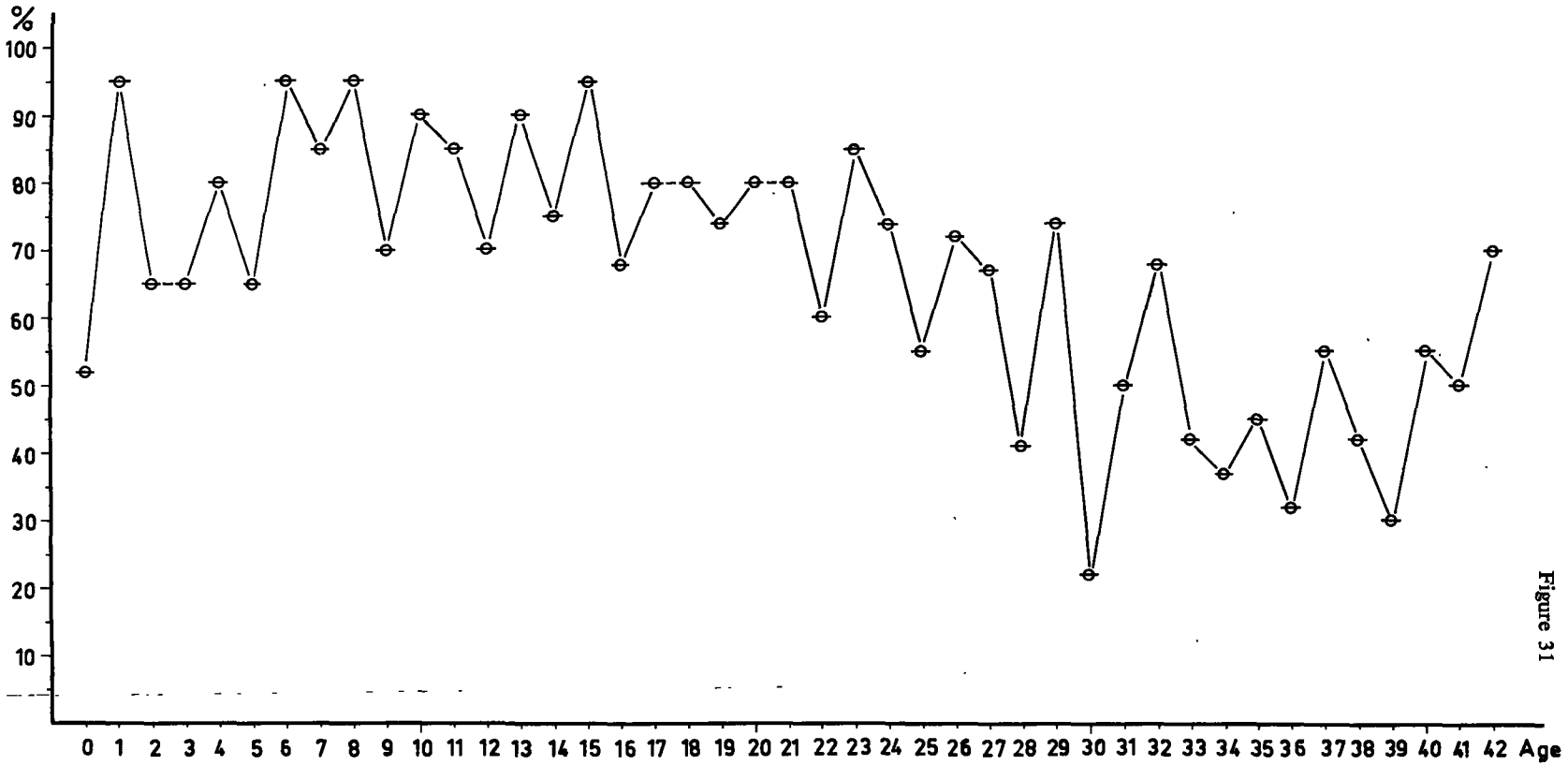

Figure 31

Figure 31. Percentage frequency of H.I. antibody for the strain of human influenza virus A2-305 (1957 Japan) in the age groups 0—42 (sera from summer 1958; about 20 sera per age group).

TABLES

Table 1. Titration of the homologous ferret antiserum, and the normal serum of the same ferret, against the strain of influenza virus A1 (1949 Nederland). Both sera were treated with 2-fold dilutions of cholera-filtrate in agar filtrate (5 parts).

Dilution of cholera-filtrate	Dilution of homologous ferret antiserum (No. 520)												Titre
	9	18	36	72	144	288	576	1152	2304	4608	9216	18432	
1/1	0	0	0	0	0	0	++	++++	++++	++++	++++	++++	775
1/2	0	0	0	0	0	0	+	++++	++++	++++	++++	++++	975
1/4	0	0	0	0	0	0	++	++++	++++	++++	++++	++++	775
1/8	0	0	0	0	0	0	+	++++	++++	++++	++++	++++	975
1/16	0	0	0	0	0	0	++	++++	++++	++++	++++	++++	775
1/32	0	0	0	0	0	0	0	0	++	++++	++++	++++	3100
1/64	0	0	0	0	0	0	0	0	++	++++	++++	++++	3100
1/128	0	0	0	0	0	0	0	0	0	0	++++	++++	8700
1/256	0	0	0	0	0	0	0	0	0	++	++++	++++	6150
1/512	0	0	0	0	0	0	0	0	0	++	++++	++++	6150
Agar-filtrate	0	0	0	0	0	0	0	0	0	0	+	++++	15500

Dilution of cholera-filtrate	Dilution of normal ferretserum (No. 520)												Titre
	9	18	36	72	144	288	576	1152	2304	4608	9216	18432	
1/1	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	< 9
1/2	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	< 9
1/4	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	< 9
1/8	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	< 9
1/16	+	++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	20
1/32	0	0	0	0	0	++	++++	++++	++++	++++	++++	++++	375
1/64	0	0	0	0	0	0	++	++++	++++	++++	++++	++++	775
1/128	0	0	0	0	0	0	0	0	++++	++++	++++	++++	2290
1/256	0	0	0	0	0	0	0	0	0	0	++++	++++	8700
1/512	0	0	0	0	0	0	0	0	0	0	++++	++++	8700
Agar-filtrate	0	0	0	0	0	0	0	0	0	++	++++	++++	6150

Table 1

Table 2. H.I. tests performed on pairs of human sera (influenza A2, 1960). Cholera filtrate treated and untreated sera were both titrated against the ferret-mouse-egg line of the strains A2-305 (1957 Japan) and A2-37 (1957 Nederland), the latter being practically non-sensitive to non-specific serum inhibitor.

Sera treated with cholera-filtrate		Untreated Sera	
A2-305 (1957 Japan) $E_5F_3M_6E_{14}$	A2-37 (1957 Ned.) $F_5M_{13}E_3$	A2-305 (1957 Japan) $E_5F_3M_6E_{14}$	A2-37 (1957 Ned.) $F_5M_{13}E_3$
$<9/80$	$<9/150$	$650/650$	$<9/150$
$<9/50$	$<9/<9$	$1350/1700$	$<9/<9$
$<9/1700$	$<9/600$	$13500/2700$	$<9/400$
$<9/<9$	$<9/<9$	$2700/1900$	$25/50$
$<9/1700$	$<9/450$	$650/5400$	$<9/600$
$<9/30$	$<9/10$	$850/650$	$<9/20$

Table 3. Difference in avidity for crossing H.I. antibody of two different laboratory lines of swine influenza virus. (A-Swine-15 (1930 U.S.A.) F_xM_xE and A-Swine-15 (1930 U.S.A.) $M_{257}E$). The ferret antisera were obtained from a reinfection experiment (1 experiment). In this and following experiments all strains used as antigen and for the preparation of antisera were adapted to mice.

Strains	H.I.-titre in ferret antisera after successive infections							
	A-PR8 (1934 U.S.A.) ×	A-PR8 (1934 U.S.A.) ×	A1 (1947 Engl.) ×	A1 (1949 Ned.) ×	A1 (1951 Engl.) (Liv.) ×	A1 (1951 Ned.) (Sc.) ×	A1 (1953 Ned.) ×	A1 (1956 Ned.)
A-Swine-15 (1930 U.S.A.) $F_x M_x E_{61}$	<9	<9	<9	15	60	100	60	80
A-Swine-15 (1930 U.S.A.) $F_x M_x E_{64}$	<9	<9	<9	<9	40	60	25	40
A-Swine-15 (1930 U.S.A.) $M_{257} E_2$	<9	<9	125	200	1150	2900	1450	900
A-Swine-15 (1930 U.S.A.) $M_{257} E_3$	<9	<9	125	200	1450	1850	1650	1450

Table 4. Titration of a cholera-filtrate treated ferret antiserum employing the strain A-Swine-15 (1930 U.S.A.). Two-fold dilutions of the antiserum were made in normal ferret serum.

Dilution of hom. antiserum in normal ferretserum	Dilution of serum												Titre
	9	18	36	72	144	288	576	1152	2304	4608	9216	18432	
1/1	0	0	0	0	0	0	0	0	0	0	0	++++	17400
1/2	0	0	0	0	0	0	0	0	0	0	++++	++++	8700
1/4	0	0	0	0	0	0	0	0	0	++++	++++	++++	4350
1/8	0	0	0	0	0	0	0	0	++++	++++	++++	++++	2200
1/16	0	0	0	0	0	0	+	++++	++++	++++	++++	++++	975
1/32	0	0	0	0	0	0	++++	++++	++++	++++	++++	++++	550
1/64	0	0	0	0	0	++++	++++	++++	++++	++++	++++	++++	275
1/128	0	0	0	0	++++	++++	++++	++++	++++	++++	++++	++++	125
1/256	0	0	0	++++	++++	++++	++++	++++	++++	++++	++++	++++	70
1/512	0	++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	24
1/1024	0	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	17
1/2048	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	10
1/4096	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	< 9
Normal ferretserum	++++	++++	++++										< 9

Table 5. Cross H.I. tests, with 9 strains of swine influenza virus A, which were isolated in the period 1930—1957 in the U.S.A. The ferret antiserum marked with adj. b was prepared using an adjuvant vaccine (2 experiments).

Ferret Antisera	Strains								
	A-Swine-15 (1930 U.S.A.) F M E	A-Swine-15 (1930 U.S.A.) F M E M E	A-Swine-15 (1930 U.S.A.) M E	A-Swine-23 (1935 U.S.A.) M E	A-Swine-29 (1937 U.S.A.) M E	A-Swine-30 (1940 U.S.A.) M E	A-Swine (1946 U.S.A.) M E	A-Swine (1957 Wis.) E	A-Swine (1957 Wis.) E M E
A-Swine-15 (1930 U.S.A.) F M E	**2150**	800	11600	30	600	1150	700	90	170
A-Swine-15 (1930 U.S.A.) F M E M E adj. b	3800	**7600**	13000	3200	4850	6450	7700	2700	3850
A-Swine-15 (1930 U.S.A.) M E	550	250	**9200**	<9	750	400	250	40	60
A-Swine-23 (1935 U.S.A.) M E	100	2900	600	**2900**	700	2300	750	500	350
A-Swine-29 (1937 U.S.A.) M E	125	125	2900	40	**1900**	400	700	250	250
A-Swine-30 (1940 U.S.A.) M E	500	3200	3650	1150	3850	**9200**	2700	1350	1350
A-Swine (1946 U.S.A.) M E	250	300	1150	75	750	800	**1500**	950	750
A-Swine (1957 Wis.) E	125	200	450	400	500	750	1350	**12200**	10900
A-Swine (1957 Wis.) E M E	400	400	1150	125	1200	700	600	1150	**1350**

Table 6. H.I. test employing antisera from ferrets against different strains of human influenza virus A and A1, and strains of swine influenza virus A isolated in the period 1930—1957 in the U.S.A. The ferret antisera marked with adj. a and adj. b were prepared using different adjuvant vaccines.

Ferret Antisera	Strains									
	A-Swine-15 (1930 U.S.A.) F M E	A-Swine-15 (1930 U.S.A.) F M E M E	A-Swine-15 (1930 U.S.A.) M E	A-Swine-23 (1935 U.S.A.) M E	A-Swine-29 (1937 U.S.A.) M E	A-Swine-30 (1940 U.S.A.) M E	A-Swine (1946 U.S.A.) M E	A-Swine (1957 Wis.) E	A-Swine (1957 Wis.) E M E	Homologous strains
A-WS (1933 Engl.) F M E	40	40	<9							4350
A-WS (1933 Engl.) F M E adj. a	<9	<9	40	<9	200		<9	10		60800
A-WS (1933 Engl.) F M E adj. b	<9	<9	25	<9	350	<9	<9	<9	250	21500
A-PR8 (1934 U.S.A.) F M E	<9	<9	<9							17500
A-PR8 (1934 U.S.A.) F M E adj. a	25	<9	300	<9	20		170	10		20400
A-PR8 (1934 U.S.A.) F M E adj. b	<9	<9	25	<9	<9	15	75	<9	<9	26100
A1 (1947 Engl.) E	<9	<9							<9	8050
A1 (1947 Engl.) E adj. b	<9	<9	<9	100	300	180	150	125	150	60800

A1 (1947 Engl.) E M E	<9	<9	50						<9	1450
A1 (1947 Engl.) E M E adj. a	20	<9	150	10	50		50	20	25	21700
A1 (1947 Engl.) E M E adj. b	15	15	600	25	85	100	100	40	75	52100
A1 (1949 Ned.) E	25	55	45						<9	1750
A1 (1949 Ned.) E adj. a			50						<9	4250
A1 (1949 Ned.) E adj. b	<9	<9	110	<9	15	15	40	<9	10	46400
A1 (1949 Ned.) E M E	<9	<9								36800
A1 (1949 Ned.) E M E adj. a	<9	<9	<9	<9	10		20	<9		3800
A1 (1949 Ned.) E M E adj. b	<9	<9	15	<9	<9	<9	10	<9	<9	21700
A1 (1951 Engl.) (Liv.) E	<9	<9								36400
A1 (1951 Engl.) (Liv.) E adj. b	<9	<9	<9	<9	<9	<9	<9	<9	<9	18400

Table 6 continued

Table 6 (continued). H.I. test employing antisera from ferrets against different strains of human influenza virus A and A1, and strains of swine influenza virus A isolated in the period 1930—1957 in the U.S.A. The ferret antisera marked with adj. a and adj. b were prepared using different adjuvant vaccines.

Table 6 continued

Ferret Antisera	Strains									
	A-Swine-15 (1930 U.S.A.) F M E	A-Swine-15 (1930 U.S.A.) F M E M E	A-Swine-15 (1930 U.S.A.) M E	A-Swine-23 (1935 U.S.A.) M E	A-Swine-29 (1937 U.S.A.) M E	A-Swine-30 (1940 U.S.A.) M E	A-Swine (1946 U.S.A.) M E	A-Swine (1957 Wis.) E	A-Swine (1957 Wis.) E M E	Homologous strains
A1 (1951 Engl.) (Liv.) E M E	<9	<9								60800
A1 (1951 Engl.) (Liv.) E M E adj. a	<9	<9	30	<9	<9		20	<9		26000
A1 (1951 Engl.) (Liv.) E M E adj. b	<9	<9	30	<9	<9	60	30	<9	<9	14600
A1 (1951 Ned.) (Sc.) E	<9	<9	<9						<9	250
A1 (1951 Ned.) (Sc.) E adj. a			50						70	3850
A1 (1951 Ned.) (Sc.) E adj. b	125	90	150	60	85	200	170	100	200	1500
A1 (1951 Ned.) (Sc.) E F M E	<9	<9								6800
A1 (1951 Ned.) (Sc.) E F M E adj. a	<9	<9	<9	<9	<9		<9	<9		9200
A1 (1951 Ned.) (Sc.) E F M E adj. b	<9	<9	<9	<9	<9	<9	<9	<9	<9	43400

A1 (1953 Ned.) E	<9	<9								4600
A1 (1953 Ned.) E adj. b.	<9	<9	<9	<9	<9	<9	<9	<9	<9	7600
A1 (1953 Ned.) E M E	<9	<9								1150
A1 (1953 Ned.) E M E adj. a.	<9	<9	<9	<9	<9		<9	<9		7600
A1 (1953 Ned.) E M E adj. b.	<9	<9	<9	<9	<9	<9	<9	<9	<9	52100
A1 (1956 Ned.) E	<9	<9	<9	<9	<9	<9	<9	<9	<9	7450
A1 (1956 Ned.) E M E	<9	<9	<9							8500
A1 (1956 Ned.) E M E adj. a.	<9	<9	<9	<9	60		60	30		3250
A1 (1956 Ned.) E M E adj. b.	<9	<9	15	20	90	125	150	90	100	30700

Table 6 continued

Table 7. H.I. tests employing antisera from ferrets against strains of swine influenza vir
influenza virus A and A1. The three ferret antisera marked with adj. a, adj. b, and adj. c we

Ferret Antisera	Strains					
	A-WS (1933 Engl.) F M E	A-PR8 (1934 U.S.A.) F M E	A1 (1947 Engl.) E	A1 (1947 Engl.) E M E	A1 (1949 Ned.) E	A1 (1949 Ned. E M E
A-Swine-15 (1930 U.S.A.) F M E	<9	250	<9	<9	<9	<9
A-Swine-15 (1930 U.S.A.) F M E	<9	125		<9		<9
A-Swine-15 (1930 U.S.A.) F M E	<9	70	<9	<9	<9	<9
A-Swine-15 (1930 U.S.A.) F M E M E	<9	15	<9	25	<9	15
A-Swine-15 (1930 U.S.A.) F M E M E adj. a	<9	<9		10		<9
A-Swine-15 (1930 U.S.A.) F M E M E adj. b	<9	10	<9	30	<9	20
A-Swine-15 (1930 U.S.A.) M E	<9	40	<9	<9	<9	<9
A-Swine-15 (1930 U.S.A.) M E	<9	60	<9	<9	<9	10
A-Swine-15 (1930 U.S.A.) M E adj. c	40	100	<9	30	20	<9
A-Swine-23 (1935 U.S.A.) M E	<9	<9	15	50	<9	<9
A-Swine-29 (1937 U.S.A.) M E	<9	<9	<9	<9	<9	<9
A-Swine-30 (1940 U.S.A.) M E	<9	125	20	25	<9	<9
A-Swine (1946 U.S.A.) M E	<9	<9	<9	<9	<9	<9
A-Swine (1957 Wis.) E	<9	<9	<9	15	<9	<9
A-Swine (1957 Wis.) E M E	<9	<9	<9	<9	<9	<9

lated in the period 1930—1957 in the U.S.A., and also employing different strains of human epared using different adjuvant vaccines.

Strains								
A1 951 Engl.) (Liv.) E	A1 (1951 Engl.) (Liv.) E M E	A1 (1951 Ned.) (Sc.) E	A1 (1951 Ned.) (Sc.) E F M E	A1 (1953 Ned.) E	A1 (1953 Ned.) E M E	A1 (1956 Ned.) E	A1 (1956 Ned.) E M E	Homologous strains
<9	<9	<9	<9	<9	<9	<9	<9	1150
	<9		<9		<9		<9	1850
<9	<9	<9	<9	<9	<9	<9	<9	2150
<9	<9	<9	<9	<9	<9	<9	<9	7300
	<9		<9		<9		<9	6550
<9	<9	<9	<9	<9	<9	<9	<9	7600
<9	<9	<9	<9	<9	<9	<9	<9	1900
<9	<9	<9	<9	<9	<9	<9	<9	9200
<9	20	<9	<9	20	50	20	<9	12000
<9	<9	<9	<9	<9	<9	<9	<9	2000
<9	<9	<9	<9	<9	<9	<9	<9	1900
<9	<9	<9	<9	<9	<9	<9	<9	9200
<9	<9	<9	<9	<9	<9	<9	<9	1500
<9	<9	<9	<9	<9	<9	<9	<9	12200
<9	<9	<9	<9	<9	<9	<9	<9	1350

Table 8. H.I. antibody in ferret antisera for the strain of swine influenza virus A and the human strains of influenza virus A, A1, and A2. The ferrets were successively infected with the human strains with intervals of 3 weeks in between. The titrations employing the swine virus and the human A2 virus were done in one experiment.

Strains	H.I.-titre in ferret antisera after successive infection								
	A-WS (1933 Engl.)	× A-WS (1933 Engl.)	× A-PR8 (1934 U.S.A.)	× A1 (1947 Engl.)	× A1 (1949 Ned.)	× A1 (1951 Engl.) (Liv.)	× A1 (1951 Ned.) (Sc.)	× A1 (1953 Ned.)	× A1 (1956 Ned.)
A-Swine-15 (1930 U.S.A.)	70	110	1450	800	400	400	300	300	1150
A2-305 (1957 Japan)	<9	<9	<9	<9	<9	<9	<9	<9	<9
Homologous	2900	3250	2300	350	350	3850	1850	3250	1850
A-Swine-15 (1930 U.S.A.)	<9	<9	100	450					
A2-305 (1957 Japan)	<9	<9	<9	<9					
Homologous	4600	3250	1450	200					

Table 9. H.I. antibody in ferret antisera for the strain of swine influenza virus A and the human strains of influenza virus A, A1 and A2. The ferrets were successively infected with the human strains with intervals of 3 weeks in between. The titrations employing the swine virus and the human A2 virus were done in one experiment.

Strains	H.I.-titre in ferret antisera after successive infection							
	A-PR8 (1934 U.S.A.)	× A-PR8 (1934 U.S.A.)	× A1 (1947 Engl.)	× A1 (1949 Ned.	× A1 (1951 Engl.) (Liv.)	× A1 (1951 Ned.) (Sc.)	× A1 (1953 Ned.)	× A1 (1956 Ned.)
A-Swine-15 (1930 U.S.A.)	<9	<9	<9	15	15	30	100	70
A2-305 (1957 Japan)	<9	<9	<9	<9	<9	<9	<9	<9
Homologous	2900	1650	600	500	2700	1350	1650	350
A-Swine-15 (1930 U.S.A.)	<9	30	40	60	60	50	40	50
A2-305 (1957 Japan)	<9	<9	<9	<9	<9	<9	<9	<9
Homologous	6500	2900	450	300	950	500	400	150
A-Swine-15 (1930 U.S.A.)	<9	<9	120	250	1050	2700	1050	1900
A2-305 (1957 Japan)	<9	<9	<9	<9	<9	<9	<9	<9
Homologous	2900	5800	750	600	5400	1350	800	500

Table 10. H.I. antibody in ferret antisera for the strain of swine influenza virus A, present after successive infections with 3 strains of human influenza virus (A and A1) with intervals of 3 weeks in between. The titrations were done in one experiment. All sera failed to show H.I. antibody for the strain A2-305 (1957 Japan).

Strains of			H.I.-titre against A-Swine-15 (1930 U.S.A.) after		
Primary Infection	Reinfection 1	Reinfection 2	Primary Infection	Reinfection 1	Reinfection 2
A-WS (1933 Engl.) ×	A-PR8 (1934 U.S.A.) ×	A1 (1947 Engl.)	15	12	24
			30	45	45
			12	45	95
A-WS (1933 Engl.) ×	A-PR8 (1934 U.S.A.) ×	A1 (1949 Ned.)	<9	15	30
			<9	12	85
			<9	30	680
A-WS (1933 Engl.) ×	A-PR8 (1934 U.S.A.) ×	A1 (1951 Engl.) (Liv.)	20	30	60
			<9	45	85
			9	35	290
A-WS (1933 Engl.) ×	A-PR8 (1934 U.S.A.) ×	A1 (1956 Ned.)	<9	15	15
			<9	15	60
			<9	40	60

Table 11. Cross reinfection experiments in ferrets employing the strain of influenza virus A-Swine-15 (1930 U.S.A.) and 3 strains of human influenza virus (A and A1).

Strains of		Number of ferrets showing recall ($\geq 4\times$)	Mean geometric titre of ferret antisera showing recall ($\geq 4\times$)	
Primary Infection	Reinfection		A-Swine-15 (1930 U.S.A.)	Human strains
A-Swine-15 (1930 U.S.A.)	× A-PR8 (1934 U.S.A.)	$^{6}/_{6}$	$^{1100}/_{12000}$	$^{<9}/_{900}$
A-PR8 (1934 U.S.A.)	× A-Swine-15 (1930 U.S.A.)	$^{11}/_{11}$	$^{<9}/_{5550}$	$^{550}/_{8550}$
A-Swine-15 (1930 U.S.A.)	× A1 (1949 Ned.)	$^{7}/_{7}$	$^{400}/_{7500}$	$^{<9}/_{1550}$
A1 (1949 Ned.)	× A-Swine-15 (1930 U.S.A.)	$^{9}/_{9}$	$^{<9}/_{4950}$	$^{300}/_{3350}$
A-Swine-15 (1930 U.S.A.)	× A1 (1956 Ned.)	$^{5}/_{7}$	$^{800}/_{10800}$	$^{<9}/_{4100}$
A1 (1956 Ned.)	× A-Swine-15 (1930 U.S.A.)	$^{10}/_{10}$	$^{<9}/_{5650}$	$^{450}/_{10200}$

Table 12. Titres of H.I. antibody for the strain of swine influenza virus A and strains of human influenza virus (A, A1 and A2) present in ferrets after vaccination with an adjuvant vaccine, containing a mixture of the human strains A and A1.

Ferret Antisera	Strains									
	A-Swine-15 (1930 U.S.A.)	A-WS (1933 Engl.)	A-PR8 (1934 U.S.A.)	A1 (1947 Engl.)	A1 (1949 Ned.)	A1 (1951 Engl.) (Liv.)	A1 1951 Ned.) (Sc.)	A1 (1953 Ned.)	A1 (1956 Ned.)	A2-305 (1957 Japan)
414	180	18400	18400	21500	14000	27000	17000	13500	5800	<9
415	<9	2700	4600	10700	3250	7600	4600	3850	1450	<9
418	125	11600	13000	10700	7300	7600	7300	4850	1850	<9
420	<9	1600	2900	3250	3250	5400	3250	5400	800	<9
424	450	6400	5800	11600	5800	21500	13000	10700	2300	<9
425	125	1450	23200	13000	6450	37000	13000	21500	900	<9

Table 13. Titres of H.I. antibody for the strain of human influenza virus (A2-305 Japan) in sera from ferrets which were successively infected with different strains of influenza virus (swine A, human A and A1). The interval between each infection was 3 weeks (1 experiment).

Strains	H.I.-titre in ferret antisera after successive infections									
	A-Swine-29 (1937 U.S.A.) ×	A-Swine-29 (1937 U.S.A.) ×	A-WS (1933 Engl.) ×	A-PR8 (1934 U.S.A.) ×	A1 (1947 Engl.) ×	A1 (1949 Ned.) ×	A1 (1951 Engl.) (Liv.) ×	A1 (1951 Ned.) (Sc.) ×	A1 (1953 Ned.) ×	A1 (1956 Ned.)
A2-305 (1957 Japan)	<9	<9	<9	<9	<9	<9	<9	<9	<9	<9
Homologous	1650	1850	500	250	50	200	600	1450	600	400
A2-305 (1957 Japan)	<9	<9	<9	<9	<9	<9	<9	<9	<9	<9
Homologous	2300	1800	1350	1350	350	600	1400	3250	450	750

Table 14. Cross reinfection experiments in ferrets employing the strains of human influenza virus A, A1 and A2, and the strain of swine influenza virus.

Strains of		Number of ferrets showing recall (≧ 4×)	Mean geometric titre of ferret antisera showing recall (≧ 4×)	
Primary Infection	Reinfection		A2-305 (1957 Japan)	Other strains
A2-305 (1957 Japan)	× A-Swine-15 (1930 U.S.A.)	$3/9$	$85/500$	$<9/1700$
A-Swine-15 (1930 U.S.A.)	× A2-305 (1957 Japan)	$6/7$	$<9/4900$	$400/3300$
A2-305 (1957 Japan)	× A-PR8 (1934 U.S.A.)	$0/8$	—	—
A-PR8 (1934 U.S.A.)	× A2-305 (1957 Japan)	$0/5$	—	—
A2-305 (1957 Japan)	× A1 (1949 Ned.)	$2/8$	$200/1150$	$<9/900$
A1 (1949 Ned.)	× A2-305 (1957 Japan)	$0/10$	—	—
A2-305 (1957 Japan)	× A1-39 (1956 Ned.)	$9/16$	$200/2850$	$<9/1400$
A1-39 (1956 Ned.)	× A2-305 (1957 Japan)	$2/6$	$<9/2250$	$400/2300$

Table 15. Cross reinfection experiments in ferrets employing the strain of horse influenza virus A, the strain of swine influenza virus A, and the strains of human influenza virus A1 and A2.

Strains of		Number of ferrets showing recall ($\geq 4\times$)	Mean geometric titre of ferret antisera showing recall ($\geq 4\times$)	
Primary Infection	Reinfection		A-Praha-1956	Other strains
A-Praha-1956	× A-Swine-15 (1930 U.S.A.)	$^{0}/_{11}$	—	—
A-Swine-15 (1930 U.S.A.)	× A-Praha-1956	$^{1}/_{6}$	$^{<9}/_{3250}$	$^{1250}/_{6100}$
A-Praha-1956	× A1-39 (1956 Ned.)	$^{4}/_{11}$	$^{450}/_{5400}$	$^{<9}/_{8200}$
A1-39 (1956 Ned.)	× A-Praha-1956	$^{4}/_{10}$	$^{<9}/_{4200}$	$^{300}/_{6150}$
A-Praha-1956	× A1-47 (1956 Ned.)	$^{0}/_{7}$	—	—
A1-47 (1956 Ned.)	× A-Praha-1956	$^{2}/_{11}$	$^{<9}/_{1050}$	$^{300}/_{2100}$
A-Praha-1956	× A2-305 (1957 Japan)	$^{0}/_{11}$	—	—
A2-305 (1957 Japan)	× A-Praha-1956	$^{0}/_{9}$	—	—

Table 16. Height of H.I. antibody for different strains of influenza virus A found in 3 human sera with errors in labelling of the donor's age (pattern I). After obtaining new specimens, the pattern II was found.

Age	Experiment	Strains									
		A-Swine-15 (1930 U.S.A.)	A-WS (1933 Engl.)	A-PR 8 (1934 U.S.A.)	A1 (1947 Engl.)	A1 (1949 Ned.)	A1 (1951 Engl.) (Liv.)	A1 (1951 Ned.) (Sc.)	A1 (1953 Ned.)	A1 (1956 Ned.)	A2-305 (1957 Japan)
R, 24 years	I	336	<9	<9	—	<9	<9	—	<9	<9	<9
	II	15	15	271	—	220	288	—	204	102	51
L, 18 years	I	576	119	119	144	<9	36	229	15	30	<9
	II	<9	36	72	363	288	363	288	181	72	144
G, 14 years	I	168	—	<9	—	29	—	—	—	<9	—
	II	<9	<9	<9	102	144	204	144	66	26	59

Table 17. Absorption tests with human sera of different age groups (1961) using the swine influenza virus as absorbing antigen. Numerator = number of sera from which antibody was absorbed. Denominator = total number of sera showing H.I. antibody for the different strains before absorption.

Age group	Number of sera	Strains							
		A-Swine-15 (1930 U.S.A.)	A-WS (1933 Engl.)	A-PR8 (1934 U.S.A.)	A1 (1949 Ned.)	A1 (1951 Engl.) (Liv.)	A1 (1951 Ned.) (Sc.)	A1 (1953 Ned.)	A1 (1956 Ned.)
20—29	15	7/7	2/5	2/15	1/10	2/12	1/11	3/10	2/8
30—34	18	14/14	4/8	4/17	5/8	3/10	5/10	3/6	2/4
35—39	20	19/19	3/5	12/17	3/9	4/12	2/9	5/11	2/5
40 and upwards	17	17/17	9/9	9/11	12/13	14/14	10/11	4/5	4/4