INTRANASAL VIRULENCE OF PNEUMOCOCCI FOR MICE

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It is generally agreed that pneumococci associated with severe infections of human beings, for example lobar pneumonia, are for the most part antigenically specific and stable (1–6), although rough variants have been reported to occur in the sputum and lung puncture material in some cases of pneumonia (7, 8). Little was known, however, of the stability of pneumococci obtained from healthy persons prior to the observations on small groups of individuals carried out with the aid of Cooper's sera in this laboratory (9, 10). Here it was found that successive strains of pneumococci obtained from a given carrier were for the most part serologically specific, similar and stable in virulence and other characteristics in so far as could be determined in the host. These studies on small groups, confirmed later by Gundel's observations on somewhat larger numbers of persons (11) led us to the view that most strains of pneumococci behave in nature as biological entities with different specific characters which in man are relatively unchanging; but that they do differ among themselves in inherent capacity to incite natural disease, persist in tissues at the normal portal of entry, and spread from host to host (9).

Further evidence on these points has been sought by direct experiments; that is, by intranasal titration of pneumococci in mice.

This procedure, foreshadowed by Stillman's method of injecting mice with ethyl alcohol and spraying them with suspensions of pneumococci (12), and Lange's observations on mice given inhalations of pneumococci (13), developed out of previous experience in this laboratory with intranasal infection of mice with Friedländer-like bacilli (14). Recently Neufeld and Etinger-Tulczynska (15) and Brunzema (16) have reported that undiluted cultures of pneumococci instilled into the nostrils of healthy mice brought about septicemia and death in a few cases.

One advantage can be claimed for the intranasal method of pneumococcus infection in mice—that it employs what is presumably the

normal atrium of infection of pneumococci into the host, the upper respiratory tract, and elicits many of the epidemiological features of the natural infection in man. Nevertheless, since the mouse is at best an unnatural host for the pneumococcus, experimental findings with this technique are subject to a limited interpretation.

Experiments dealing with the intranasal virulence for mice of pneumococci freshly isolated from man are described in the present paper.

Technique

Rockefeller Institute albino mice, free of intercurrent infection, aged 3 months, weighing 16 to 18 gm. each, kept in a special breeding room, and maintained on a diet of bread, milk, and dog biscuit, were used in these experiments. Pneumococci for testing were obtained from sputum or nasopharynx of cases of lobar pneumonia and from the spinal fluid of cases of meningitis usually within 48 hours of admission to the hospital. Material was also obtained from the nasopharynx of healthy persons and spread over the surface of blood agar plates. The sputum or blood broth growth from the nasopharynx culture plates was injected intraperitoneally into mice, and pneumococci obtained later from the heart's blood in pure culture for testing by seeding into pneumococcus broth. Intranasal and intraperitoneal titrations were run on fresh cultures, usually within 72 hours after isolation. For the intranasal test a standard dilution of 1:100 of an 18 hour pneumococcus broth culture was used and administered in 0.03 cc. volume to each mouse, approximately 150,000 organisms. The material was placed at the orifices of the nasal passages of each individual through a 1 cc. tuberculin syringe and blunt needle. The twenty treated animals generally employed for each test were then placed in individual glass jars to prevent reinfection from contact. Animals found dead were autopsied and cultured for the presence of pneumococci. Suitable colonies grown on rabbit blood agar plates were tested in bile and type sera kindly supplied by Miss Cooper (6). Results were expressed as per cent dying of the infection within 14 days. For the intraperitoneal test, dilutions of an 18 hour broth culture from 10^{-1} to 10^{-7} were prepared and given in 1 cc. volumes to mice as follows: 10^{-1} to 10^{-5} each to one mouse, 10^{-6} and 10^{-7} each to two mice. Results were expressed in terms of the exponent of dilution of culture fatal to mice within 4 days.

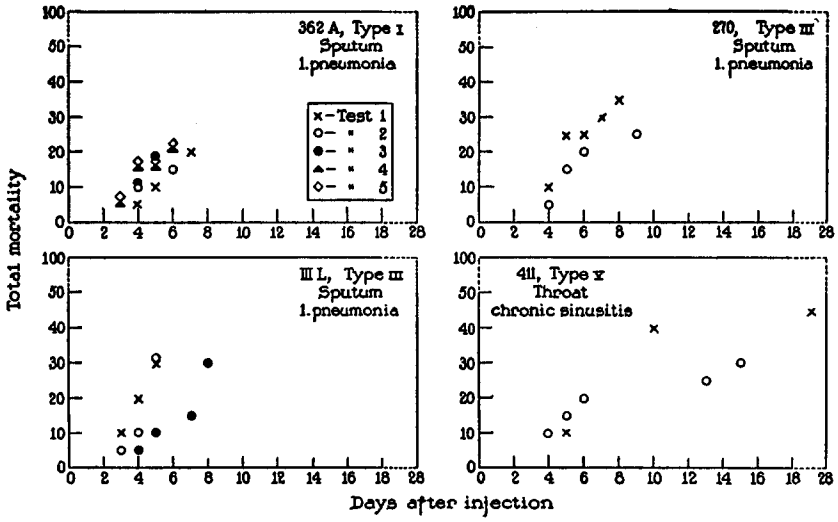
The presence of pneumococci in the nasal passages of mice was detected by instilling physiological saline into the nares, waiting until it was aspirated and ejected, and then spreading it over the surface of freshly prepared 5 per cent rabbit blood agar plates. The plates were examined after 24 hours' incubation for the presence of pneumococcus-like colonies. These were then selected and identified serologically.

Tests of the ability of pneumococci to spread from experimentally infected individuals to contacts were made by placing ten mice infected intranasally into a single cage 25 x 18 x 12 cm. in dimensions and adding ten normal mice 6 to 48

hours later, and one or two additional normal mice each day thereafter. Carrier tests were made on the entire population at frequent intervals, and autopsies and bacteriological tests on mice dying during the period of observation.

EXPERIMENTS

Early experiments showed that if a given fresh strain of pneumococcus was administered intranasally to mice of different hereditary and environmental experiences, the percentage mortalities in duplicate and repeated titrations were irregular and unpredictable, and again, if the test culture was a stock transfer from rabbit blood agar or broth



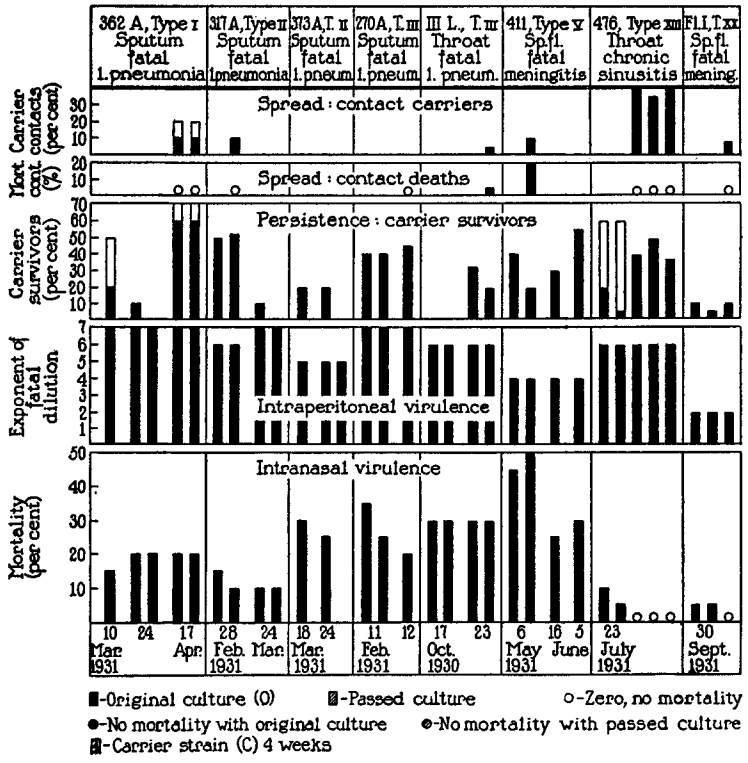
TEXT-FIG. 1

to blood broth, the mortalities dropped quickly to zero. If, however, the culture was a growth from the blood of a mouse dead of the infection, and the test mice were kept uniform as far as possible, duplicate titrations and titrations repeated at short intervals gave reliable measurements of certain potencies of pneumococci in mice.

Experiment 1.—Tests of intranasal and intraperitoneal virulence, carrier rates in survivors, and ability to set up the carrier state in contacts or to kill them have been made on 24 fresh strains. Protocols of eight are set forth briefly in Text-figs. 1 and 2.

Four strains described were from the sputum and one from the throat of fatal cases of lobar pneumonia; two were from the spinal fluid of fatal cases of meningitis; one was from the throat of a case of chronic sinusitis. One was Type I; two Type II; two Type III; one Type V; one Type XIII; one Type XX.

Each strain, when placed at the nares of twenty mice, was capable of inducing in some a fatal respiratory tract and general infection. After an incubation period of 2 days, a percentage of mice became ruffled, dyspneic, lost appetite and normal



TEXT-FIG. 2

activity, and after 3 to 7 days succumbed to the infection (Text-fig. 1). Blood cultures became positive in fatal cases within 24 hours and contained increasing numbers of pneumococci until death. Some strains persisted in the nasal passages of survivors for at least 4 weeks. The pathology of the infection was septicemia, pneumonia, pleurisy, empyema, pericarditis, and cervical lymphadenitis.[1]

[1] A detailed report of gross and histological changes will be given in the near future.

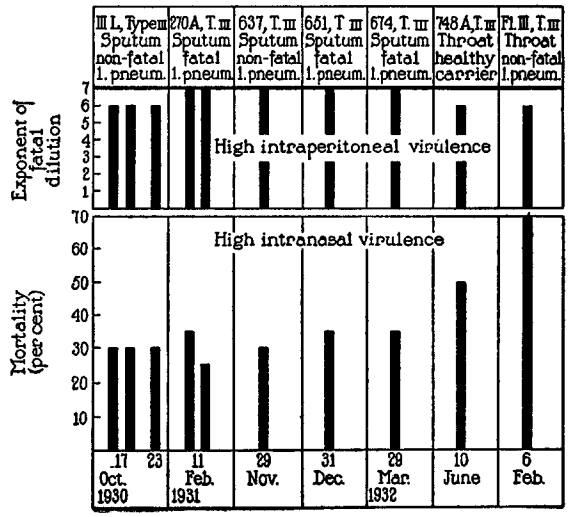
The findings leave no doubt that pneumococci are able by themselves, without the aid of any other microorganism or virus agent, unless some unrecognized form is present in the test mice or cultures, to incite in mice, under stated conditions, certain phenomena of pneumococcus infection.

The titration results indicated (Text-fig. 2), moreover, that a satisfactory control of variables had been achieved, since intranasal tests run in duplicate or repeated at short intervals, resulted usually in mortalities which approximated each other within 10 per cent. An exception is shown in the protocol of Strain 411, in which the mortalities over a 2 weeks period differed by 20 per cent. In these instances the routine method for preserving the virulence of the culture probably did not suffice.

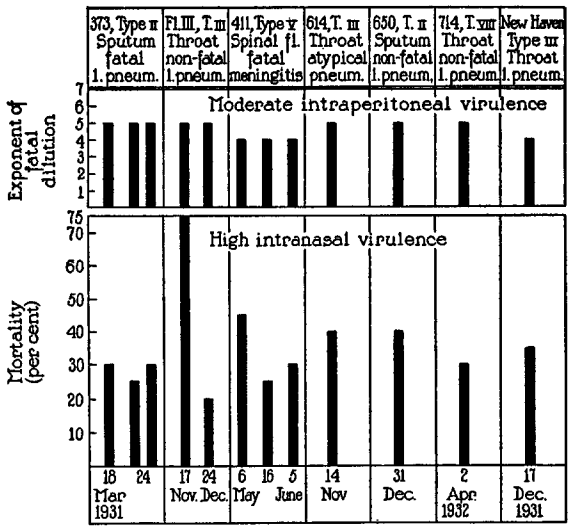
The results indicate also that individual mice of a given test batch differ profoundly in their response to pneumococci. Although all were reared under similar conditions, without previous exposure, and were given the same dose of the same culture at the same time under circumstances in which all known variables were controlled, some proved refractory; some survived and became nasal carriers; others developed cervical adenitis which was usually fatal; others, fatal pneumonia with empyema and pericarditis; and finally, others, fatal septicemia and death within 72 hours. These differences and others brought out in histopathological studies to be described later were apparent even when the undiluted culture was employed and are ascribable not to chance but to individual differences in inherent resistance factors.

The tests brought out a parallelism between degree of intranasal virulence and tendency to persist in survivors and spread to contacts to the extent that strains which spread to contacts were invariably of high intranasal virulence and strains which set up the carrier state in survivors were generally of moderate or high intranasal virulence.

Finally, the strains displayed individualities with respect to their intranasal virulences and abilities to persist and spread to contacts. Some exhibited high intranasal and low intraperitoneal virulences—No. 411,—others, low intranasal and high intraperitoneal virulences—No. 317A,—while still others showed intranasal and intraperitoneal virulences running parallel—No. 270A. Some, but by no means all



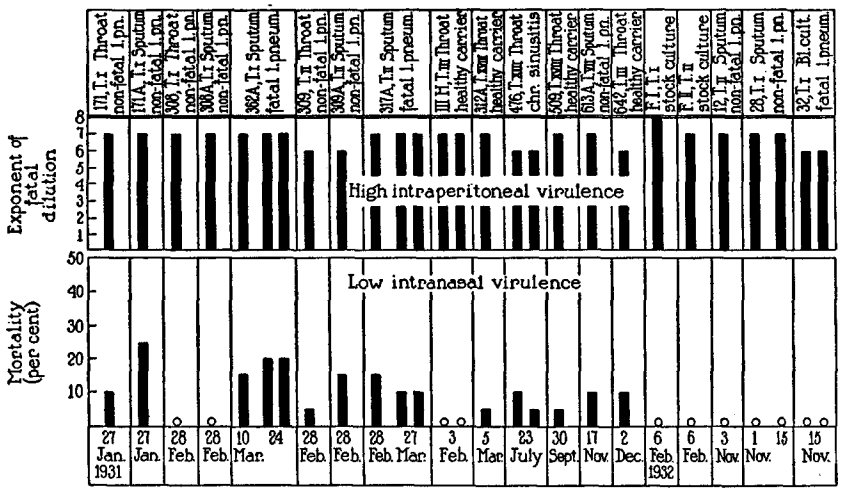
TEXT-FIG. 3*a*



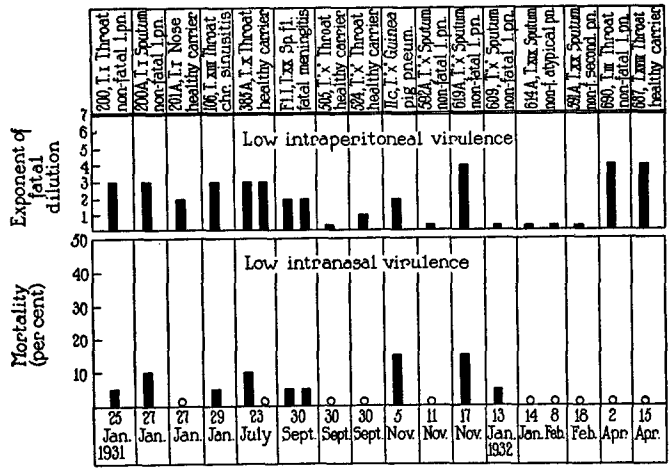
TEXT-FIG. 3*b*

strains spread from infected to contact mice, inciting in them in turn the carrier state or fatal infection.

The individualities of pneumococci with respect to infective potencies in mice brought out in the previous experiment were analyzed



TEXT-FIG. 3*c*



TEXT-FIG. 3*d*

further by comparing intranasal and intraperitoneal virulences of the twenty-four strains discussed above and an additional twenty-six strains.

Experiment 2.—Of the total 50 strains, eleven were Type I, ten from cases and one from a healthy carrier; seven were Type II from cases; fourteen were Type III, eleven from cases and three from carriers; eighteen were other named and unnamed types, nine from cases and nine from carriers. As stated before, strains from cases were obtained usually within 48 hours of admission to the hospital and strains from carriers directly from the throats of healthy persons. All strains were of the so called "smooth colony" variety. Simultaneous intranasal and intraperitoneal tests were run with the same culture on twenty and ten mice respectively, as described above, sometimes in duplicate, sometimes at short or occasionally long intervals.

The results of 69 intranasal titrations on a total of 1,380 mice and 69 simultaneous intraperitoneal titrations of 690 mice on the 50 strains summarized in Text-fig. 3 bear on three main points. First,

TABLE I

Classification of Serological Types of Pneumococci According to Intranasal and Intraperitoneal Virulence

Type of pneumococcus	No. of strains	Per cent with high intranasal, high intraperitoneal virulence	Per cent with high intranasal, moderate intraperitoneal virulence	Per cent with low intranasal, high intraperitoneal virulence	Per cent with low intranasal, low intraperitoneal virulence
I	11	—	—	72.7	27.3
II	7	—	28.5	71.5	—
III	14	57.1	21.4	14.3	7.2
Others	18	—	11.1	22.2	66.6

there were repeated demonstrations of the ability of certain strains of pneumococci to incite a characteristic picture of a natural infection. Second, there was additional evidence of the individuality of strains with respect to intranasal virulence. Eight were classed as high in intranasal, 38.3 ± 3.8 (standard error) per cent and high in intraperitoneal, 6.5 ± 0.08 virulences; seven as high in intranasal, 31.2 ± 2.2 per cent, and moderate in intraperitoneal, 4.7 ± 0.07 virulences; nineteen as low in intranasal, 7.4 ± 1.5 per cent, and high in intraperitoneal, 6.8 ± 0.03 virulences; and sixteen as low in intranasal, 3.9 ± 1.2 per cent, and low in intraperitoneal, 1.8 ± 0.34 per cent virulences. One exceptional strain, No. 171A, with high intraperitoneal virulence, might be placed either in the high or low intranasal groups. It was included in the latter with most of the other Type I strains.

Third, there were positive and negative relationships between amount of intranasal virulence of strains and other properties. Thus, intranasal virulence did not parallel degree of intraperitoneal virulence in 50 per cent of cases. Moreover, strains of both high and low intranasal virulences formed smooth colonies suggesting lack of complete relationship between intranasal virulence and S substance. Again, degree of intranasal virulence did not appear to be related to source of strain in humans. Of the eleven Type III strains from cases, ten were of high intranasal virulence, while of three Type III strains from the nasopharynx of healthy persons, one was of high intranasal virulence.[2] A possible relationship to serological type is suggested by the data (Table I). Of eleven Type I strains, all were of low intranasal virulence, although 72 per cent were of high intraperitoneal and 27.3 per cent low intraperitoneal virulence. Of seven Type II strains, 28.5 per cent were of high intranasal and moderate intranasal virulence, and 71.5 per cent of low intranasal and high intraperitoneal virulence. Of fourteen Type III strains, 78.5 per cent were of high intranasal virulence—91 per cent of Type III strains from cases belonged in this category. And finally, of eighteen other named and unnamed strains, only 11.1 per cent showed high intranasal virulences. In brief, Type I strains were of low intranasal virulence; Type II strains low or high; Type III strains high; and strains of other types were generally low. The possible significance of these relationships will be discussed later.

Titrations of sputum and throat strains from the same person and different strains of the same type obtained at the same time from the same person gave comparable results.

The remainder of this report deals with the stability of intranasal virulence of pneumococci for mice. Pneumococci are perhaps the classic example of bacteria pathogenic for human beings, whose virulence by animal test is regarded as labile. This view is based on the fact that repeated intraperitoneal passage in mice of a laboratory Neufeld Type I strain, the pathogenicity of which has dropped so low that 100,000,000 organisms injected intraperitoneally fail to kill, will in certain cases have the consequence that as few as ten organisms prove

[2] Eighteen additional carrier Type III strains obtained from sixteen young adults over a 5 weeks period did not differ significantly in intranasal virulence from case Type III strains isolated and titrated at about the same time.

fatal by the intraperitoneal route. These and similar experiments with streptococci and other organisms have been given broad epidemiological interpretation. Opposed to these data, however, are experiments with bacteria native to the test host in which freshly isolated strains are administered by way of the normal portal of entry in doses comparable with those in nature. Under these conditions, the manifestations of the natural infections are observed under controlled circumstances and virulence has proved relatively stable (17).

The opposing views have now been tested by comparing the stability of what might be called relatively natural, that is intranasal virulence, and artificial or intraperitoneal virulence of fresh strains of pneumococci. The test is incomplete in that the test animal, the mouse, is essentially a foreign host. To this extent, therefore, not only the intraperitoneal test, but the intranasal test as well, must be regarded as unnatural.

The first series of experiments dealt with the effect of the passage of pneumococci from host to host by way of the nose on both intranasal and intraperitoneal virulences.

Experiment 3.—A series of passages was made by transferring repeatedly a blood culture from a mouse dying of the infection to the nasal passages of healthy mice.

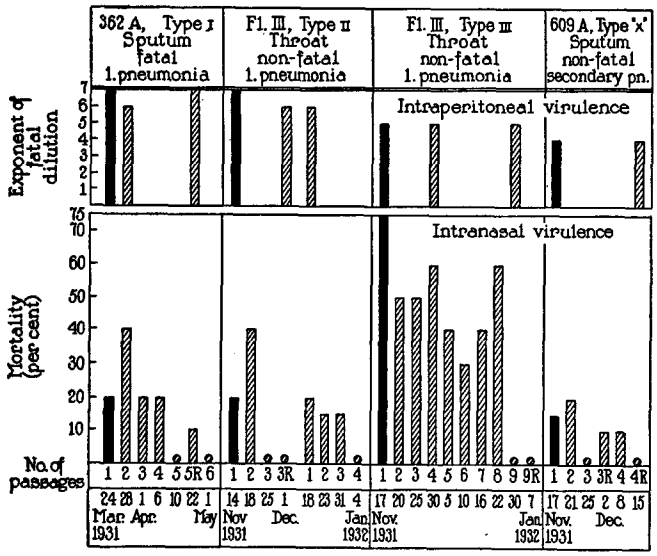
Nineteen strains were employed; two Type I, five Type II, ten Type III, and two other named types from cases of lobar pneumonia. The strain, first seeded into pneumococcus broth from the blood of a mouse dying after intraperitoneal injection of the organisms, was diluted 1:100 and given in 0.03 cc. quantities intranasally to ten or twenty mice according to the usual routine. From the heart's blood of the first mouse to die following the intranasal instillation, a culture was taken, seeded to broth, diluted, and given intranasally as before to a second batch of mice. This procedure was repeated routinely. Intraperitoneal virulence tests were done on the unpassed and suitable passed cultures. Carrier tests were made on survivors at various intervals after exposure.

Protocols of tests with four strains are given in Text-fig. 4. Passage led invariably to a sudden drop in intranasal virulence from a level characteristic of the strain to zero. This drop occurred after few or many passages, depending on the strain tested. No change in the intraperitoneal virulence of the passage culture was detected. The smooth type of colony on blood agar and agglutinative proper-

ties were apparently unaltered. Persistence of the organisms as measured by carrier rates did not change materially with passage nor with the drop of intranasal virulence to zero.

Experiment 4.—In this series, pneumococci were passed from the nasal passages of surviving carriers to the nasal passages of unexposed healthy individuals.

The following procedure was carried out on four strains; one Type I, one Type II, and two Type III. The test strain was injected into the peritoneal cavity of a



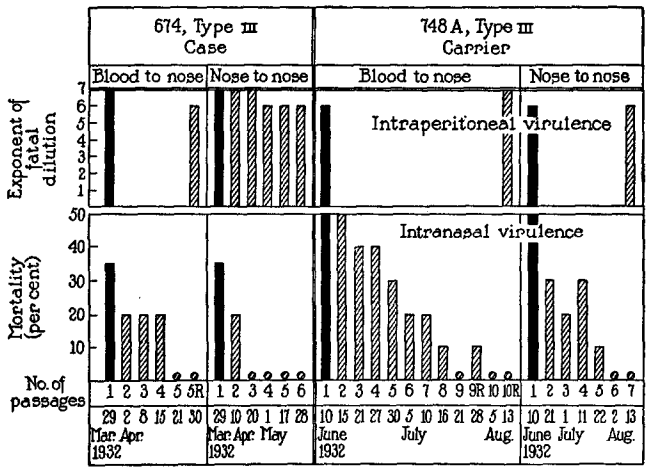
TEXT-FIG. 4

mouse and recovered 24 hours later from the dead animal's heart blood. The culture, grown 8 hours in blood broth, was diluted as usual and given intranasally to twenty mice. Approximately 2 weeks later, carrier tests were done on survivors, a pneumococcus colony from a positive plate transferred to broth, incubated 18 hours, diluted, and administered intranasally to ten mice. This procedure was repeated. As a control, case to case passages, as described in Experiment 3, were run simultaneously with the same strain. That is, from the first mouse to die of the first group infected intranasally, a blood culture was obtained and given intranasally to a second group. This was continued in series.

The results of intranasal passage of two strains are shown in Text-fig. 5.

In each instance, passage of pneumococcus from nose to nose resulted in a drop in its intranasal virulence from its characteristic level to zero. Indeed, the drop occurred more quickly when the culture was passed *via* this relatively normal route than when it was passed from blood of fatal cases to nasal passages of healthy mice. Intraperitoneal virulence, however, was not altered. Persistence, as measured by carrier rates, was apparently not affected. Smooth type colony and agglutinative characteristics remained apparently unchanged.

Experiment 5.—Strains of pneumococci were not only passed intranasally but were tested after a sojourn of several weeks in the nasal passages of an individual.



TEXT-FIG. 5

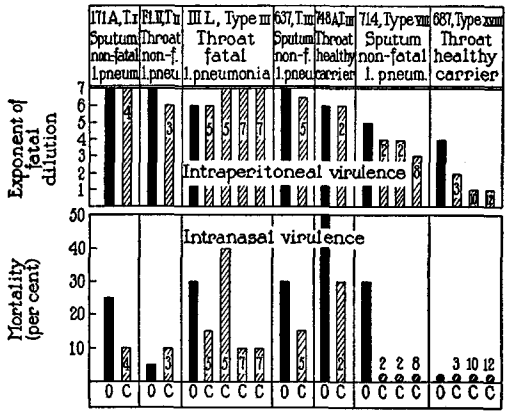
A given strain was instilled into the nasal passages of mice in the routine manner. 2 to 12 weeks later, carrier tests were made on survivors and cultures from positive cases were seeded into blood broth, incubated 18 hours, diluted, and given intranasally to a test group of twenty mice. The results were compared with those of a similar titration made with the freshly isolated strain given the usual single peritoneal passage. Intraperitoneal virulences of carrier strains were also determined and compared with those of the freshly isolated strain.

The results of tests on fourteen carrier cultures from seven strains, one Type I, one Type II, three Type III, and two other named types, are given in Text-fig. 6. In no instance was the intranasal virulence

of a carrier culture significantly greater than that of the original strain; on the contrary, it was usually markedly decreased. Intraperitoneal virulence, smooth colony form, agglutinative, and other properties were apparently unaltered.

To summarize briefly, passage of pneumococci through mice by way of the nose led consistently to a loss of intranasal virulence; such passage, however, did not change the intraperitoneal virulence, nor colony and agglutinative properties.

Finally, passages were made by transfers of mouse heart's blood cultures without cultivation on media to peritoneal cavity of mice.



TEXT-FIG. 6

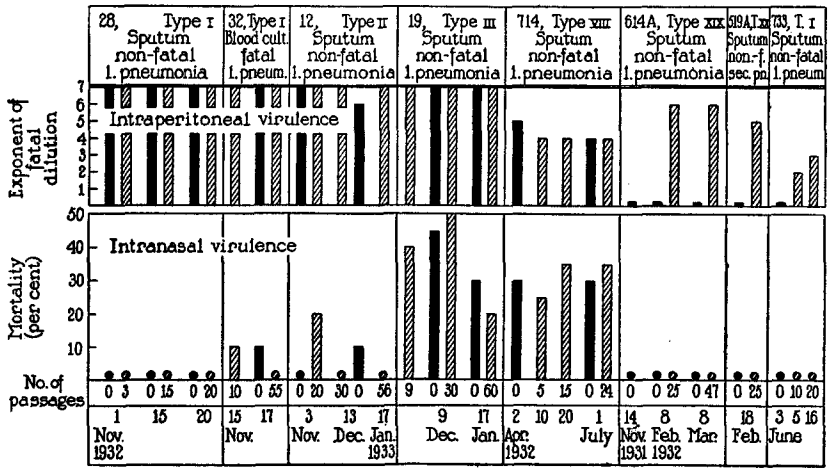
Under these conditions certain laboratory strains are known to increase in intraperitoneal virulence.

Experiment 6.—Serial intraperitoneal passages were carried out on three Type I strains, one Type II, four Type III, and one each of Types VIII, XIX, XX, and X. These were all smooth colony forms freshly isolated from human cases of lobar pneumonia.

Each test culture was injected into the peritoneum of a mouse and transferred shortly before or after the death of the animal by removing the heart, cutting it into small bits aseptically, and washing it with 2 cc. of pneumococcus broth. This broth containing pneumococci from the heart's blood was then immediately injected in 1 cc. quantities into the peritoneum of two mice. This passage procedure was continued routinely every 24 hours. At suitable intervals comparative titrations were made of passed and unpassed cultures. The passed culture was

taken from the heart's blood of a mouse dead following intraperitoneal injection, placed in pneumococcus broth, and grown 18 hours in the incubator; the unpassed culture was taken from the stock blood agar slant, injected into the peritoneum of a mouse, transferred from its heart's blood shortly before or after death to pneumococcus broth and incubated 18 hours. The numbers of organisms per cubic centimeter in the unpassed and passed cultures were found to be similar. Both were diluted in the routine manner and administered intranasally and intraperitoneally into the usual batches of twenty and ten mice respectively.

The results of these tests are summarized in Text-fig. 7. The first four strains, Nos. 28, 32, 12, and 19, were initially high in intraperitoneal virulence. No. 28, Type I, was tested on three occasions by simultaneous titrations of unpassed



TEXT-FIG. 7

and passed cultures: Nov. 1, 1932, three passages; Nov. 15, 1932, fifteen passages; Nov. 20, 1932, twenty passages. In each instance the intraperitoneal virulence was maximum, 10^{-7}, and the intranasal virulence zero. Strain 32, Type I, was tested twice: once on Nov. 15, 1932, when the ten passage strain showed an intraperitoneal virulence of 10^{-7} and an intranasal virulence of 10 per cent, and again on Jan. 17, 1933, after 55 passages, when simultaneous titrations of unpassed and passed cultures showed intraperitoneal virulences of 10^{-7} and intranasal virulences of 10 per cent and 0 per cent respectively. Strain 12, Type III, was tested three times: once on Nov. 3, 1932, with simultaneous titrations of unpassed and twenty passage cultures showing intraperitoneal virulences of 10^{-7} and intranasal virulences of 0 per cent and 20 per cent respectively; again on Dec. 13, 1932, when the unpassed culture showed an intraperitoneal virulence of 10^{-6} and an in-

tranasal virulence of 10 per cent, and the thirty passage culture an intraperitoneal virulence of 10^{-7} and intranasal virulence of 0 per cent; and a third time on Jan. 17, 1933, when the 56 passage culture showed an intraperitoneal virulence of 10^{-7} and an intranasal virulence of 0 per cent. Strain 19, Type III, was tested three times,—once on Nov. 1, 1932, when the nine passage culture showed an intraperitoneal virulence of 10^{-7} and an intranasal virulence of 40 per cent; again on Dec. 9, 1932, when the unpassed and thirty passage cultures showed intraperitoneal virulences of 10^{-7} and intranasal virulences of 45 per cent and 50 per cent respectively; and finally on Jan. 17, 1933, when the 60 passage culture showed an intraperitoneal virulence of 10^{-7} and an intranasal virulence of 20 per cent. The other four strains were initially low in intraperitoneal virulence. Strain 714, Type VIII, showed unpassed on Apr. 2, 1932, an intraperitoneal virulence of 10^{-5} and an intranasal virulence of 30 per cent. On June 5, 1932, the five passage culture showed an intraperitoneal virulence of 10^{-4} and an intranasal virulence of 25 per cent; on June 20, 1932, the fifteen passage culture an intraperitoneal virulence of 10^{-4} and intranasal virulence of 35 per cent; and finally, on July 1, 1932, the unpassed and twenty-four passage cultures, an intraperitoneal virulence of 10^{-4} and intranasal virulences of 30 per cent and 35 per cent respectively. Strain 614 A, Type XIX, showed on Nov. 14, 1931, and Feb. 8, 1932, the unpassed culture to be of 1 cc. intraperitoneal and 0 per cent intranasal virulences. On Feb. 8, 1932, a twenty-five passage culture showed an intraperitoneal virulence of 10^{-6} and an intranasal virulence of 0 per cent; on Mar. 8, 1932, simultaneous tests showed the unpassed culture to be of 1 cc. intraperitoneal and 0 per cent intranasal virulences and the forty-seven passage culture of 10^{-6} intraperitoneal and 0 per cent intranasal virulences. Strain 591 A, Type XX, was tested on Feb. 18, 1932, after twenty-five passages. The unpassed culture showed an intraperitoneal virulence of 1 cc. and an intranasal virulence of 0 per cent; the passage culture an intraperitoneal virulence of 10^{-5} and an intranasal virulence of 0 per cent. Strain 733, Type I, showed an intraperitoneal virulence of the unpassed culture on June 3, 1932, of 1 cc. and an intranasal virulence of 0 per cent; on June 5, 1932, the ten passage culture had increased in intraperitoneal virulence to 10^{-2}, but its intranasal virulence remained at 0 per cent; and on June 16, 1932, the twenty passage strain, when injected intraperitoneally, killed in a dilution of 10^{-3}, but failed to kill when given intranasally.

In brief, fresh strains of pneumococci from human cases of initially high or low intraperitoneal virulence, when passed through mice by intraperitoneal injections, did not increase in intranasal virulence but maintained their characteristic level. Some strains did show an increase from low to high intraperitoneal virulence. No changes in colony form or agglutinative properties were noted.

Two fresh strains from healthy carriers gave similar results.

DISCUSSION

The nasal instillations described in this report were made with freshly isolated strains of smooth colony forms of pneumococci from different sources under rigidly standardized conditions, such as are essential in this type of work. The technique reproduced normal conditions in so far as small doses, portal of entry, and setting up of natural infections are concerned, and was artificial in that pneumococci in nature do not ordinarily infect mice. The data bear on special questions of pneumococcus infection and more general questions of epidemiology.

The first question relative to pneumococcus infection is whether the pneumococcus in nature is a primary or secondary invader. The time-honored belief in its essential pathogenicity (18, 19) is being questioned in the light of the realization that pneumococci occur without harm in the nasopharynx of healthy persons, that they do not by themselves readily incite lobar pneumonia in the experimental animal, and that, like influenza bacilli and other organisms, they may give rise to more typical experimental infection when associated with a virus. The present experiments, however, confirming and amplifying previous work (12–16), support the older view in demonstrating that pneumococci are capable by themselves,—unless indeed the action of some attendant virus with unique, unknown properties is hypothesized,—of inciting a natural infection in mice, persisting in the nasopharynx of survivors, and spreading to contacts.

The next point to be noted is that any given strain of pneumococcus evoked different responses in different individual mice and in different batches from known susceptible or resistant stocks (20), ranging from the refractory or carrier state to acute septicemia and death. This series of differences compares closely with those described in native mouse typhoid (21) and rabbit and fowl Pasteurella infections (22, 23) especially, in which all variables save those referable to the host were apparently controlled. The finding agrees with the recent observations of Goodner and Stillman (24) on intracutaneous infections of rabbits with pneumococci, indicating that degree of infection depends as much upon host as bacterial properties (17).

Third, the experiments bear out the observational data on human

beings as concerns the individuality of strains of pneumococci of the same or different types (9, 10). Under the conditions specified, strains have shown determinate and characteristic pathogenic properties with no evidence of colony dissociation or serological type transformations.

Fourth, a certain lack of relation between degree of nasal virulence of pneumococci and amount of specific substance present, already commented on by others who have encountered smooth colony strains with no intraperitoneal mouse virulence (25), is suggested by the facts that the intranasal virulence of smooth colony strains may be high or low irrespective of intraperitoneal virulence, and drops to zero when strains are passed through mice by intranasal instillations, without affecting intraperitoneal virulence or smooth colony form.

Fifth, to the extent that intranasal titrations measure the natural virulence of pneumococci for mice, Type III strains must be regarded as more virulent than Type II, and Type II more virulent than Type I; but whether or not intranasal titrations in mice measure the natural virulence of pneumococci for human beings is a matter for conjecture. True, the intranasal method shows in the case of Types I, II, and III strains relative mouse mortalities which are consistent with human pneumonia mortalities. But since mortalities of human cases must reflect uncontrolled host as well as virulence factors (26), there is in reality very little basis for comparison.

Two findings of general epidemiological significance bearing on the question of stability of bacterial virulence are that the intranasal virulence of pneumococci for mice was in no case enhanced, and instead of remaining stable dropped to zero when the organisms were passed by way of the nose. Failure to increase intranasal virulence of pneumococci (even under conditions in which the intraperitoneal virulence was enhanced) is in agreement with experience with other bacteria tested in their native host under relatively natural conditions (17). Failure to maintain the characteristic virulence of pneumococci, the phenomenon of its total loss, is the first exception encountered by us to the general finding that native virulence is relatively stable. It is referable in all probability to the fact that in nature pneumococci do not readily infect and hence that we are not actually dealing with native virulence.

CONCLUSIONS

1. Smooth colony pneumococci fresh from human beings, instilled in small doses into the nasal passages of special mice raised under standard conditions, brought about a characteristic infection and this spread to healthy contacts inciting in them the carrier state or fatal infection.

2. Differences in individual host response to the same dose of a given culture ranged from a complete refractory or nasopharyngeal carrier state, or a local cervical lymphadenitis, to fatal lobular or lobar pneumonias with or without pleurisy, empyema, and pericarditis, and acute fatal septicemia.

3. Pneumococci exhibited consistent individual strain differences with respect to ability to infect, when instilled intranasally into mice, and also differences in the spread to contacts. Degree of intranasal virulence paralleled demonstrable ability to spread to contacts.

4. Degree of intranasal virulence of strains did not parallel intraperitoneal virulence in 50 per cent of strains—high intranasal was accompanied by either high or moderate intraperitoneal virulence, and low intranasal by high, moderate, or low intraperitoneal virulence.

5. Type III strains were of relatively high intranasal and intraperitoneal virulences; Type II strains mostly low in intranasal but high or moderate in intraperitoneal virulence; Type I strains all low in intranasal but either high or moderate in intraperitoneal virulence. Most strains of other types were low both in intranasal and intraperitoneal virulences.

6. The intranasal virulence of pneumococci was not enhanced by animal passage. Nasal passage reduced the intranasal virulence to zero but did not affect intraperitoneal virulence, colony form, and agglutinative specificity. Passage by the intraperitoneal method maintained the characteristic level of intranasal virulence for a period, increased intraperitoneal virulence in some instances, but did not affect colony form or agglutinative properties.

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