STUDIES ON HAEMOPHILUS INFLUENZAE*

I. Infection of Mice with Mucin Suspensions of the Organism

By LeROY D. FOTHERGILL, M.D., JOHN H. DINGLE,** Sc.D., and CAROLINE A. CHANDLER, M.D.

(From the Department of Bacteriology and Immunology, and the Department of Pediatrics, The Harvard Medical School, Boston)

(Received for publication, February 8, 1937)

Investigations of the immunologic problems concerned in infections with *Haemophilus influenzae*, the most important and serious of which is meningitis, have been hindered to a very considerable extent by the lack of an experimental animal with adequate susceptibility to infection by this organism. Studies dealing with the development of a specific form of treatment would be facilitated to a considerable extent if a true infection could be established in a laboratory animal.

Many investigators (1–3) in the past have attempted to produce infections in animals with *H. influenzae*. While it is possible to kill laboratory animals with sufficiently large doses of the organism, it cannot be said that a true infection, characterized by *in vivo* multiplication of organisms, has ever been demonstrated. It is likely that death has occurred in most instances simply from the toxic effects of such large doses. Various animals, including monkeys, rabbits, guinea pigs, and mice have been used in such studies and all react in a similar manner. It is obviously difficult to pursue experimental studies of such problems as active and passive immunization and the development of methods for standardizing and appraising the related biological products, under these circumstances.

Nungester, Wolf, and Jourdonais (4) first demonstrated the effect of mucin on the mouse virulence of the pneumococcus, streptococcus, and staphylococcus and later, Miller (5) found that mice, normally resistant, can be infected by mucin suspensions of the meningococcus. It seemed possible, therefore, that mucin might also be utilized to produce an infection in mice with the influenza bacillus.

The experiments[1] to be described in this communication show that: (*a*) the suspension of *H. influenzae* in mucin enhances the power of this

* Aided by the Philip Ellis Stevens, Jr., Memorial Fund.

** James Jackson Cabot Fellow.

[1] All strains of *H. influenzae* used in these experiments were isolated from the cerebrospinal fluid of cases of meningitis, were smooth in character and serologically homogeneous in type (presumably the type b of Pittman).

organism to infect mice; (*b*) *in vivo* multiplication of the organism with subsequent death of the animal occurs; (*c*) repeated mouse passage appears to enhance the "mucin virulence;" and (*d*) protection tests and passive immunization experiments can be carried out with this method.

Experiments Which Establish the Occurrence of a True Infection in Mice

The evidence which appears to establish the occurrence of a true infection in mice with *H. influenzae* suspended in mucin was obtained by employing the following methods.

Mucin suspensions were prepared according to the techniques described by Miller (6) and more recently by Nungester, Jourdonais, and Wolf (7), using granular mucin, type 1701-W.[2] In all experiments, except the one otherwise designated, mucin prepared according to the method of Miller was used.

The organism was grown on peptic digest blood agar plates as described in a previous paper (8) for 10 to 18 hours at 37°C. Usually the seed inoculum was the heart blood of an infected mouse, evenly spread over the surface of the plate with a capillary spreader. The growth, which generally was confluent, was carefully scraped up in 1.5 cc. of sterile infusion broth, yielding a dense well suspended preparation. Broth was used for making suspensions and dilutions in order to avoid possible toxic effects of physiological salt solution. In addition to observing the plates carefully for contaminating colonies, purity of the suspension was determined by examining stained smears and subcultures on peptic digest-blood agar plates. Smears of these young cultures of virulent organisms exhibited no clumping and a minimum of pleomorphism.

In preparing mucin suspensions for mouse inoculation, the above broth suspension was diluted 1:4 with mucin. From this mixture further dilutions in mucin were carried on decimally using a separate sterile pipette for making and mixing adequately each dilution. For inoculation of control mice, as well as for plate counts, a similar series of dilutions was prepared in sterile infusion broth. An intraperitoneal dose of 1 cc. of the resulting mucin or broth suspensions was employed. In most instances, 6 mice were inoculated with each dilution: 4 received the suspension in mucin, and 2 the control suspension in broth. Such an experiment is illustrated in Table I.

It is apparent that mice receiving mucin suspensions of the organism succumbed to a much smaller infecting dose than did those receiving the corresponding broth suspension—in this case, the majority of mice inoculated with the 1–40,000 dilution in mucin died, whereas all control mice survived beyond a dilution of 1–4 in broth.

[2] Purchased from the Wilson Laboratories, Chicago, Illinois.

The character of the infection produced in mice was as follows:

Usually no symptoms were exhibited for several hours after inoculation. The animal then gradually developed evidences of a severe infection, *i.e.*, refusal of food, ruffling of fur, diarrhea, and lassitude. These symptoms increased progressively until death; they were not, however, specific in character. The majority of mice died between 18 and 36 hours, although occasional animals survived for as long as 72 hours. In all instances, mice dying even on the 3rd day showed positive cultures at autopsy. Mice living longer than 72 hours rarely succumbed to the infection. Histopathological studies have not yet been made to determine

TABLE I

The Effect on Mouse Virulence of Suspending H. influenzae in Mucin

Dilution of organisms*	Mucin suspension		Broth suspension	
	D	S	D	S
1–4	4	0	1	1
1–40	4	0	0	2
1–400	4	0	0	2
1–4,000	4	0	0	2
1–40,000	3	1	0	2
1–400,000	0	4	0	2
1–4,000,000	0	4	0	2
1–40,000,000	0	4	0	2

* *H. influenzae*, F. strain, isolated from the cerebrospinal fluid of a case of meningitis.

D, died.

S, survived.

the extent and character of any pathological processes occurring in the mice infected by this method.

At autopsy, the peritoneal exudate was variable in amount, seropurulent in character, and occasionally mucoid. Smears of such exudate usually exhibited large numbers of *H. influenzae* and many leucocytes, a preponderance of which were polymorphonuclear cells. When a virulent organism was used, very few bacteria were phagocytized. A greater degree of phagocytosis was observed following the inoculation of an avirulent organism. Depending on certain circumstances the organisms appeared as large swollen, globoid bodies, as described by Pittman (3). A more detailed study of these forms will be reported in a subsequent paper.

Cultures of both heart blood and peritoneal exudate yielded extraordinarily

large numbers of organisms. They were so plentiful in the heart blood as to be easily seen by direct smear. Furthermore, the swollen forms mentioned above could be demonstrated in smears of the heart blood. Centrifugated or filtered peritoneal washings gave a positive precipitin test with immune serum, thus indicating the presence of free precipitinogen, presumably soluble specific substance. This reaction was demonstrable even after a considerable dilution, as is evident from Table II.

TABLE II

Precipitin Reactions with Peritoneal Washings from Mice Succumbing to H. influenzae Infection

Mouse No.	Dilution of peritoneal washings								
	Undiluted	1-2	1-4	1-8	1-16	1-32	1-64	1-128	Control
1	++	++	++	++	++	++	+	+	0
2	++	++	++	++	++	+	+	0	0
3	++	++	++	++	+	+	0	0	0
4	++	++	++	+	0	0	0	0	0
5	++	++	++	++	++	+	0	0	0

The peritoneal washing of each mouse was made up to a volume of 6 cc. with physiological salt solution, centrifugated at high speed, and the supernatant fluid diluted as indicated before layering over anti-*H. influenzae* horse serum. Readings were made after 30 minutes at room temperature.

TABLE III

Invasion of Blood Stream Following Intraperitoneal Injection of H. influenzae

Mouse No.	Amount of peritoneal washing injected with 1 cc. mucin	Colonies in 1 drop of tail blood			
		15 min.	30 min.	45 min.	60 min.
	cc.				
D 70	0.5	38	300±	+++	+++
D 71	0.25	2	147	121	+++
D 72	0.1	5	52	83	+++

Invasion of the blood stream with *H. influenzae*, as determined by plating a drop of tail blood at 15 minute intervals after inoculation, occurred with great rapidity following intraperitoneal injection of mucin suspensions of the organism. This is illustrated in Table III.

The data presented above constituted no evidence that multiplication of the organism occurred in the infected mouse, although such

appeared very probable. Positive evidence on this point would be obtained if direct mouse to mouse passage of the organism could be carried out for an appreciable number of transfers. In order to determine this, the following experiment was performed.

Normal mice were inoculated with a lethal dose of *H. influenzae* (strain 62) suspended in mucin. After death of the mice, either a small quantity of peritoneal washing (usually 0.1 cc. proved lethal) or 1 drop of heart blood was mixed with 1 cc. of mucin and injected intraperitoneally into a normal mouse. As soon as possible after death of this animal, the process was repeated. At the time of each

TABLE IV

*Comparison of the Effect of Two Different Mucin Preparations on the Virulence of H. influenzae for Mice**

Dilution of organisms†	Mucin suspension (Nungester)‡		Mucin suspension (Miller)§		Broth suspension	
	D	S	D	S	D	S
1–4	4	0	4	0	4	0
1–40	4	0	4	0	2	2
1–400	4	0	4	0	0	4
1–4,000	4	0	4	0	0	4
1–40,000	4	0	2	2	0	4
1–400,000	3	1	2	2	0	4
1–4,000,000	1	3	2	2	0	4
1–40,000,000	1	3	0	4	0	4

* Swiss albino strain of mice.

† *H. influenzae*, strain 62, 45th mouse passage, 11th direct mouse to mouse passage.

‡ Relative viscosity approximately 10, as determined by the Ostwald viscosimeter.

§ 5 per cent suspension of mucin.

mouse to mouse inoculation, control smears and cultures were made to prove that the inoculum had not been contaminated. In the first part of this experiment 9 successive mouse to mouse passages were obtained before temporary interruption by contamination. Later a similar series of 11 passages was carried out. It is inconceivable that this would have been possible without actual *in vivo* multiplication of the organism.

In one experiment only, infection of mice with *H. influenzae* suspended in mucin prepared according to the method of Nungester, Jourdonais, and Wolf was compared with that obtained by the use of Miller's method of preparation. From this experiment, illustrated in Table IV, it appears that the former type of suspen-

sion produces more uniform results. More detailed comparative studies are being made.

Summarizing the above experiments, it is evident that: (*a*) with the aid of mucin, fatal infections with *H. influenzae* can be produced in mice; (*b*) actual multiplication of the organism with invasion of the blood stream occurs during such infection; and (*c*) the lethal dose of *H. influenzae* for mice is greatly decreased by suspending the organism in mucin.

TABLE V

Variation in Susceptibility of Mouse Strains to H. influenzae

Dilution of organisms	Unknown stock				Albino Swiss strain				Black Swiss strain			
	Mucin		Broth control		Mucin		Broth control		Mucin		Broth control	
	D	S	D	S	D	S	D	S	D	S	D	S
1–4	7	0	3	1	8	0	4	0	8	0	4	0
1–40	7	0	3	1	8	0	4	0	8	0	4	0
1–400	5	2	1	3	8	0	1	3	8	0	0	4
1–4,000	4	3	0	4	8	0	1	3	7	1	0	4
1–40,000	1	6	0	4	4	0*	0	4	4	0*	0	4
1–400,000	0	7	0	4	5	3	0	4	1	7	0	4
1–4,000,000	0	7	0	4	1	7	0	4	0	8	0	4

* Only 4 mice were inoculated in these 2 groups.

The Importance of Standard Mouse Strains in Determinations of the Virulence of H. influenzae

Very irregular and spotted deaths were noted in our earlier experiments with stock mice purchased from various animal supply houses. Since the work of several investigators (9, 10) has established the value of inbred strains of mice for experiments of this type, two such strains were examined in the hope of finding one which would yield more consistent and uniform results.

Table V demonstrates the comparative virulence of *H. influenzae* for (*a*) a heterogeneous and unknown genetic stock purchased from dealers, (*b*) an albino Swiss strain, and (*c*) a black Swiss strain of mice.[3] Breeding of the Swiss mouse strains was carried on in this

[3] Both of the latter strains were obtained from the Army Medical School.

laboratory; all animals subsequently used for experimental purposes were descendant from an original 30 stock mice of each strain.

These results show that not only were the mice of both the albino and black Swiss strains more susceptible than those of unknown and heterogeneous stock, but also that a much sharper end-point was obtained with either of the two former strains. No significant difference was noted in susceptibility of the albino and black Swiss strains, as indicated by ultimate survival of the mice. It was observed, however, that the duration of the infection in the black mice was consistently longer, that is, such mice, although obviously sick, did not succumb in as short a period of time, possibly due to slightly greater resistance of this strain, or more probably because these mice were in general older and larger than the albino Swiss mice. Inasmuch as the black mice were also more vicious and difficult to breed, the albino strain was selected for use in the subsequent experiments.

It is, of course, impossible entirely to eliminate the occasional inconsistencies due to individual animal variation in experiments of this kind, but it is of great importance to reduce such inconsistencies to a minimum. That such can be accomplished to a considerable extent is evident from the above data obtained by the use of a standard, inbred mouse strain.

Increase in Mucin Virulence of H. influenzae by Repeated Passage through Mice

It is ordinarily difficult, if not impossible, to increase significantly the virulence of an organism for a species of animal naturally resistant to infection with that organism. That mice are normally rather resistant to *H. influenzae* is apparent from the fact that in broth suspensions large numbers of organisms are required to kill these animals. With the aid of mucin, however, a much smaller dose is lethal; the organism under this condition acquires, at least relatively, an increased virulence, which we shall term its mucin virulence for the purposes of this discussion. The following experiment was carried out to determine whether or not the mucin virulence of *H. influenzae* could be increased by repeated passage through mice, employing suspensions in mucin as the inoculum.

The strain of *H. influenzae* used, No. 62, was isolated from the cerebrospinal fluid of a case of influenza bacillus meningitis on Oct. 6, 1936. Typical smooth colonies were obtained. Supernatants of broth cultures gave a precipitin reaction with anti-*H. influenzae* horse serum in a dilution of 1–32. The virulence of this organism was determined in the manner described above, with organisms obtained from the first subculture after isolation. 45 mouse passages of this strain were carried out, the greater proportion of them being direct mouse to mouse transfer employing peritoneal washings or heart blood in mucin as the inoculum. During the occasional 2 or 3 days' interval between two series of passages, the heart's blood was cultured in whole defibrinated rabbit blood, incubated 12 to 18 hours, and stored in the ice box. Virulence tests were performed at intervals, the suspension being prepared from peptic digest blood agar plates inoculated with heart blood of the respective passage mouse and incubated for 10 hours. Bacterial counts were made in the following ways: (*a*) by plating 1 cc. of the control broth dilutions with 10 cc. of peptic digest blood agar and incubating 72 hours at 37°C., and (*b*) by carefully smearing 0.25 cc. of each dilution over the surface of a chocolate or peptic digest blood agar plate with a capillary spreader and incubating for 24 or 48 hours.

The results of seven virulence titrations are presented in Table VI.

It can be seen that after 10 passages, the mucin virulence of this organism for mice was increased approximately one hundred-fold, and that subsequent repeated mouse passage did not augment this virulence to any significant degree. On the other hand, the ability of the organism, without mucin, to kill mice was not increased.

Plate counts have indicated that the smallest number of bacteria required to kill all 4 mice varied from 340,000 organisms in the first titration to 7,800 organisms and 27,200 organisms after the 29th and 45th mouse passages, respectively. It is felt, however, that little reliance can be placed on these estimates in regard to the absolute number of organisms present, not only because of considerable differences obtained by the two methods of plate counts, but also because direct counts on a few of these suspensions have demonstrated 10 to 50 times as many bacteria as the viable counts have indicated. The fact that the conditions under which the suspensions have been prepared were kept as constant as possible, and also that the virulence titrations checked remarkably well, indicates that the minimum lethal dose is sufficiently large to be relatively unaffected in a decimal series of dilutions by moderate differences in the original suspensions.

A study of the comparative mucin virulence of a series of smooth

TABLE VI

Increase in Mucin Virulence of H. influenzae Strain 62 by Repeated Mouse Passage

Mouse passages..	0				4th				10th				17th				27th				29th				45th			
Dilution of organisms	Mucin		Broth control		Mucin		Broth control		Mucin		Broth control		Mucin		Broth control		Mucin		Broth control		Mucin		Broth control		Mucin		Broth control	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
1–4	4	0	2	0	4	0	2	0	4	0	2	0	4	0	2	0	4	0	2	0	4	0	2	0	4	0	4	0
1–40	4	0	2	0	4	0	1	1	4	0	0	2	4	0	2	0	4	0	2	0	4	0	1	1	4	0	2	2
1–400	4	0	2	0	4	0	0	2	3	1	0	2	4	0	2	0	4	0	1	1	4	0	0	2	4	0	0	4
1–4,000	2	2	0	2	4	0	0	2	4	0	0	2	4	0	0	2	4	0	1	1	3	1	0	2	4	0	0	4
1–40,000	1	3	0	2	3	1	0	2	4	0	0	2	2	2	0	2	4	0	0	2	4	0	0	2	2	2	0	4
1–400,000	1	3	0	2	1	3	0	2	3	1	0	2	1	3	0	2	3	1	0	2	2	2	0	2	2	2	0	4
1–4,000,000	0	4	0	2	0	4	0	2	1	3	0	2	2	2	0	2	1	3	0	2	1	3	0	2	2	2	0	4
1–40,000,000	0	4	0	2	0	4	0	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	4	0	4

TABLE VII

Comparative Mucin Virulence of a Series of Smooth Strains of H. influenzae

Dilution of organisms	Strain 1				Strain 3				Strain 4				Strain 5				Strain 14				Strain 15			
	Mucin		Broth control		Mucin		Broth control		Mucin		Broth control		Mucin		Broth control		Mucin		Broth control		Mucin		Broth control	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
1–4	4	0	1	1	4	0	2	0	3	1	0	2	4	0	2	0	4	0	2	0	4	0	2	0
1–40	4	0	0	2	4	0	0	2	3	1	0	2	4	0	0	2	3	1	0	2	4	0	0	2
1–400	4	0	1	1	4	0	0	2	2	2	0	2	3	1	1	1	3	1	0	2	3	1	0	2
1–4,000	4	0	0	2	4	0	0	2	0	4	0	2	1	3	0	2	2	2	0	2	0	4	0	2
1–40,000	1	3	0	2	3	1	0	2	0	4	0	2	0	4	0	2	0	4	0	2	0	4	0	2
1–400,000	0	4	0	2	1	3	0	2	0	4	0	2	0	4	0	2	0	4	0	2	0	4	0	2
1–4,000,000	1	3	0	2	1	3	0	2	0	4	0	2	0	4	0	2	0	4	0	2	0	4	0	2

strains of *H. influenzae* has been made. These strains have been maintained for a variable period of time by weekly transplants in fresh, whole, defibrinated rabbit blood. During this period, all the cultures have retained their smooth characteristics, as evidenced by colony morphology, as well as the type of growth and production of specific precipitating substances in broth culture. The results of virulence titrations in Table VII show a difference in the mucin virulence of these organisms. Strains 1 and 3 possessed a mucin virulence comparing favorably with that of the F. strain (Table I) and almost equal to that of strain 62 after repeated mouse passage. On the other hand, strains 4, 5, 14, and 15 exhibited a lower mucin virulence, comparable to that of strain 62 before mouse passage. These differences were not correlated with the duration of the period of artificial cultivation in blood, since strains 1 and 3 were isolated before the other strains of lower virulence. It is of further interest that, regardless of the mucin-virulence, there was no essential difference in the ability of the organisms to kill mice without mucin.

It would appear, therefore, that although the mucin virulence of *H. influenzae* for mice can be increased by repeated mouse passage of the organism in mucin suspension, ultimate fixation of such virulence occurs, beyond which no further increase is possible by this method. Furthermore, smooth strains suspended in mucin vary considerably in mucin virulence as compared with that of such a fixed strain, without any significant difference in their ability to kill mice in broth suspension.

Passive Immunization of Mice

The establishment of a true infection with *H. influenzae* in mice is of importance because it provides a means of determining the value of passive and active immunization. The following preliminary experiments are presented to illustrate this point.

In the first experiment, mice were injected with 0.5 cc. of anti-*H. influenzae* horse serum[4] administered intraperitoneally or intravenously 2 hours before

[4] The anti-*H. influenzae* horse serum used in these tests was obtained from a horse which has been kept under continuous active immunization for a number of years. Immunization has been maintained by repeated series of injections of formalinized suspensions of the 24 hour growth of a smooth meningeal strain. The strain used for this purpose has been replaced frequently by a freshly isolated one of the same serologic type, presumably the type b of Pittman.

inoculation with the suspensions of the organism which had been isolated from the cerebrospinal fluid of a case of meningitis (Br.). Plate counts showed the

TABLE VIII

The Mouse Protective Action of Anti-H. influenzae Horse Serum

Dilution of organisms*	Immune serum				Controls			
	0.5 cc. Anti-*H. influenzae* serum, ip		0.5 cc. Anti-*H. influenzae* serum, iv		Mucin suspension of organisms		Broth suspension of organisms	
	D	S	D	S	D	S	D	S
1–4	3	0	3	0	3	0	1	0
1–40	0	3	0	3	3	0	1	0
1–400	0	3	0	3	3	0	0	1
1–4,000	0	3	0	3	1	2	0	1
1–40,000	0	3	0	3	1	2	0	1
1–400,000	0	3	0	3	0	3	0	1

ip = intraperitoneal administration.
iv = intravenous administration.
* *H. influenzae*, Br. strain.

TABLE IX

The Mouse Protective Action of Anti-H. influenzae Horse Serum against a Lethal Test Dose of H. influenzae in Mucin

Dilution of serum	Anti-*H. influenzae* horse serum 0.5 cc. ip		Normal horse serum 0.5 cc. ip	
	D	S	D	S
Undiluted	1	5	6	0
1–4	0	6	6	0
1–16	1	5	5	1
1–64	0	6	6	0
1–256	3	3	6	0
1–1,024	5	1	4	2

All mice received 1.0 cc. intraperitoneally of 1–4,000 mucin suspension of *H. influenzae*, strain 62, 17th mouse passage. 4 additional control mice succumbed to the test dose of organisms.

original suspension, prepared in the usual manner, to contain 6,000,000,000 organisms per cc. The results, shown in Table VIII, demonstrate obvious protection.

In a second experiment, the protective action of anti-*H. influenzae* horse serum was titrated against a standard dose of organisms suspended in mucin. The organism employed was strain 62 after 17 mouse passages, suspended as described above. The virulence of the organism in this suspension (Table VI, 17th mouse passage) was such that 1 cc. of a 1–4,000 dilution killed all of 4 mice inoculated, and 2 of 4 mice succumbed with 1 cc. of the 1–40,000 dilution. Plate counts gave an estimate of 61,600,000 organisms per cc. in the original suspension. 1 cc. of 1–4,000 dilution in mucin was arbitrarily selected as a test dose and was administered intraperitoneally 2 hours after administration of the serum dilutions, which were given intraperitoneally in 0.5 cc. amounts. The results, given in Table IX, show definite protection in the mice receiving anti-*H. influenzae* horse serum, compared to those receiving normal horse serum.

The above results thus indicate that it is possible to protect mice passively against a lethal infection with *H. influenzae*. Methods of titrating more accurately the protective action of antisera are being investigated further and will be reported in a subsequent publication, together with the results of active immunization experiments.

DISCUSSION

The above results demonstrate that by the aid of mucin a fatal infection with smooth strains of *H. influenzae* can be induced in mice. Such infection is characterized by rapid invasion of the blood stream resulting in a bacteremia, and *in vivo* multiplication of the organism as demonstrated in the present instance by 11 successive mouse to mouse passages. The occurrence of a true septicemia, with multiplication of the bacilli in the blood stream, seems probable but has not been proved, since it is possible that the organism multiplies in the peritoneal cavity with secondary invasion of the blood stream.

The work of other investigators establishing the value of a homogeneous, inbred stock of mice for experiments of this kind has been confirmed. With such a stock it is possible to obtain more consistent results by reducing to a minimum, although not eliminating, spotted deaths due to individual variations in the host.

By means of this method of determining virulence, it is possible to demonstrate considerable differences in the capacity of various meningeal strains of *H. influenzae* to kill mice. Furthermore, the mucin virulence of such strains can be increased by repeated mouse passage until apparent fixation occurs. The strains examined approach this

point to a greater or less degree, some being very nearly as virulent as the fixed strain; others, considerably less so. These same strains without mucin, however, kill mice only in enormous doses, and show no significant difference in their ability in this respect—an added indication that such lethal effect is due chiefly to the inherent toxicity of the organism.

The mechanism by which mucin augments the virulence of smooth strains of *H. influenzae* is as yet undetermined. The fact, however, that this organism is presumably destroyed by complemental bacteriolysis, without phagocytosis, does not support the theory of Nungester, Jourdonais, and Wolf that mucin interferes with the bactericidal properties of phagocytic cells in destroying bacteria. The mechanism of disposal of this organism, as shown *in vitro* by Ward and Wright (11), apparently is not phagocytic but bactericidal.

Furthermore, these experiments indicate that by this method it is possible to demonstrate passive protection of mice infected with mucin suspensions of *H. influenzae*. In addition, this protective action of appropriate antisera can be titrated. Thus this method promises to be of value in assaying the potency of immune sera for clinical use. This method may also be utilized to investigate active immunization in animals.

The results of further studies made possible by the use of the mucin technique will form the subject matter of later communications in this series, *i.e.*, (*a*) the comparison of the virulence of smooth and rough strains; (*b*) the mechanism of the action of mucin; (*c*) a comparison of the susceptibility of inbred mouse strains to this organism; (*d*) the value of passive immunization in titrating the protective activity of immune sera; and (*e*) the problem of active immunization.

SUMMARY

1. It has been shown that the ability of virulent *H. influenzae* to kill mice is enhanced by suspending the organism in mucin.

2. The occurrence of a true infection characterized by *in vivo* multiplication of the organism has been established.

3. The necessity for using a pure inbred strain of mice for this type of study has been confirmed.

4. The increase in mucin virulence of a strain of *H. influenzae* by repeated passage through mice has been shown.

5. The usefulness of this method in passive immunization studies has been demonstrated.

BIBLIOGRAPHY

1. Wollstein, M., *J. Exp. Med.*, 1911, **14,** 73; 1915, **22,** 445.
2. Albert, H., and Kelman, S. R., *J. Infect. Dis.*, 1919, **25,** 433.
3. Pittman, M., *J. Exp. Med.*, 1931, **53,** 471.
4. Nungester, W. J., Wolf, A. A., and Jourdonais, L. F., *Proc. Soc. Exp. Biol. and Med.*, 1932, **30,** 120.
5. Miller, C. P., *Science*, 1933, **78,** 340.
6. Miller, C. P., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32,** 1136.
7. Nungester, W. J., Jourdonais, L. F., and Wolf, A. A., *J. Infect. Dis.*, 1936, **59,** 11.
8. Fothergill, L. D., and Chandler, C. A., *J. Immunol.*, 1936, **31,** 401.
9. Webster, L. T., *J. Exp. Med.*, 1933, **57,** 793, 819.
10. Schütze, H., Gorer, P. A., and Finlayson, M. H., *J. Hyg.*, Cambridge, Eng., 1936, **36,** 36.
11. Ward, H. K., and Wright, J., *J. Exp. Med.*, 1932, **55,** 223.