THE RÔLE OF MICROBIC VIRULENCE, DOSAGE, AND HOST RESISTANCE IN DETERMINING THE SPREAD OF BACTERIAL INFECTIONS AMONG MICE

I. Pasteurella lepiseptica and Pasteurella aviseptica Infections

By LESLIE T. WEBSTER, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

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The studies contained in this and the two following papers were made for the purpose of measuring directly the factors responsible for the spread of certain epidemic diseases. The method consisted in the analysis of microbic dosage and virulence and of host resistance in mouse populations during the pre-epidemic, epidemic, and post-epidemic phases of the infections studied. Moreover, tests were made with a view to determining whether an experimental alteration of host-resistance results in a predictable change in the extent and severity of disease. In this paper, studies on Pasteurella infections are reported; studies on a Friedländer-like bacillus infection and *B. enteritidis* infection are described in the second (Ia) and third papers (Ib) respectively.

Technique

The mice constituting each population were assembled in a single, well-ventilated cage, measuring 35 x 35 x 35 cm. in dimensions. This form of housing, by confining the specific bacteria to a limited and definite space, insured a high degree of control of the bacterial dosage factor. Each population in its respective cage was placed on a large table at relatively equal distances from light and heat sources. The routine diet consisting of pasteurized Grade "B" milk plus white bread was given daily at 8:30 to 10:00 a.m. Bedding consisted of wood shavings. The cleaning was done by transferring the mice twice a week to a sterilized cage containing fresh bedding. To each population, two normal mice with identification marks were added daily. A daily census was kept, and the animals found dead were removed at 9:00 a.m. and 5:00 p.m. These stated intervals were ad-

hered to in spite of cannibalism and partial decomposition of the bodies, because regulated removals of the dead presumably affected the numbers of specific bacteria in the cage at definite rather than random periods throughout the day. The carcasses, unless destroyed by the living mice, were autopsied and studied bacteriologically. Routine cultures were made from heart's blood, lungs, spleen, and intestines to suitable agar plates and identified later by cultural and serological tests.

The mice employed in these experiments came from The Rockefeller Institute breeding room. These mice, strain inbred for more than 15 years, have been kept in a special room where constant breeding regulations, daily routine, and dietary regime prevail. The number of deaths in this breeding stock 500 to 800, plus 500 to 800 offspring, averages about one individual per month. All dead animals are examined. The post-mortem tests may show the presence of a Pasteurella-like pneumonia in 1 to 2 pregnant females per year during the cold weather, and *B. enteritidis* infection in 2 to 3 individuals during the winter and in 8 to 10 during July and August. Numerous tests for fecal carriers have revealed the presence of *B. enteritidis* in less than 0.05 per cent. The mice used in the following experiments were approximately 12 weeks old, and weighed 16 to 18 gm. each.

The Spread of Pasteurella Infections

The first observations dealt with the primary response of a population to certain foreign bacilli. Experiment 1 was made to test the mode of spread of such strains of pathogenic Pasteurella in communities of mice previously unexposed to these organisms.

Experiment 1.—June, 1925, four populations were assembled by placing ten mice in each of four regular cages. Each received a specific culture of Pasteurella. Two fresh mice were added daily to each population.

Population 1, June 27, 1925, received a rabbit Pasteurella strain designated "Rivers G." It was a variant obtained from the "Rivers D" culture by prolonged growth in plain broth, and was relatively avirulent for rabbits (1c). 0.1 cc. of an 18 hour rabbit-blood broth culture was instilled into the nares of each mouse.

Population 2, June 11, 1925, received the fowl Pasteurella strain designated "Pa." It was obtained by Dr. Theobald Smith from an epidemic of fowl cholera and proved highly virulent and relatively non-vegetative for chickens (1d). The bacilli were administered by mixing an 18 hour blood broth culture with bread and placing it in the cage as the food ration for one day.

Population 3, June 27, 1925, received the rabbit Pasteurella strain "Rivers D." This organism was obtained from a rabbit dying of septicemia and represented the highly pathogenic and non-vegetative variety (1c). The mice received an intranasal instillation of 0.1 cc. of an 18 hour blood broth culture.

Population 4, June 27, 1925, was given a similar intranasal instillation of the rabbit Pasteurella strain, DC 30. This culture, obtained from a rabbit dead of

spontaneous pneumonia, is an example of the moderately virulent, vegetative variety common in rabbits (1c).

Text-fig. 1, which tabulates the results of the tests, is constructed as follows: The daily population is plotted as a series of dots; the average survival time of the two mice added on a given day is shown by the solid line, and the numbers of deaths on a given day are indicated by a series of rectangles. These rectangles are marked further to indicate whether or not the animal was autopsied, and if so, what the findings were.

The mortality records raise two questions which should be considered before the general results of the experiments are discussed—namely, the diagnosis and disposal of the few dead mice with negative autopsy findings, and the large number of non-autopsied mice. The following pertinent statements can be made, based on 4 years' data of over 6000 mice. Mice which presented no lesions or specific bacteria at autopsy were for the most part victims of accidental death (fighting, etc.). A few, however, may have died of one of the specific infections and failed to yield the inciting organism. Mice which were not autopsied suffered this neglect because of cannibalism, post mortem decomposition, or lapse in technique. In assigning to these latter a probable cause of death, old age can be ruled out because the known mean age at death of 2000 of these mice is many months less than the known average duration of life of the normal standard mice, and intercurrent infection can likewise be dismissed because none save those infections under investigation occurred. Since 99 per cent of the individuals subjected to autopsy yielded the specific organism, the death of non-autopsied individuals can with safety be attributed to the infections prevailing in the communities at the time. Hence for statistical purposes, all deaths have been considered as specific and in the general discussion, total mortality figures have been employed.

The reactions of mouse populations to the presence of foreign strains of Pasteurella are shown in Text-fig. 1. None of the rabbit strains survived. The "Rivers G" and DC 30 strains in Populations 1 and 4, infected none of the mice and died out at once; the "Rivers D" culture in Population 3 killed 2 of the originally inoculated mice but did not spread. However, the fowl cholera strain, "Pa," in Population 2 behaved in quite a different manner.

Eight of the ten mice originally fed with the "Pa" culture died within 2 weeks with Pasteurella septicemia. During the following 6 weeks, no further deaths from this infection occurred. However, a mouse found dead on July 21 showed Pasteurella "Pa" in blood and organ cultures, together with *B. enteritidis*.[1] This animal had been added to the population on June 27, 5 days after the last Pasteur-

[1] The appearance of *B. enteritidis* in this and other populations is discussed later (1b).

ella fatality. No further Pasteurella deaths were recognized until August 14, 24 days later, when the population numbered 61 individuals. During the succeeding 5 days, however, 47 died. On the first day, 9 succumbed and Pasteurella "Pa" was recovered from the blood and tissues of 5 subjected to autopsy; on the second day, 2 died, also yielding the "Pa" organisms; on the third day, 27 died and from each of the 25 mice autopsied, the "Pa" strain was obtained. Seven mice died on the fourth day, all giving positive cultures, and twelve on the fifth day, four of which were examined and found to contain the "Pa" organism. The epidemic then ceased abruptly and *P. avicida* disappeared from the population.

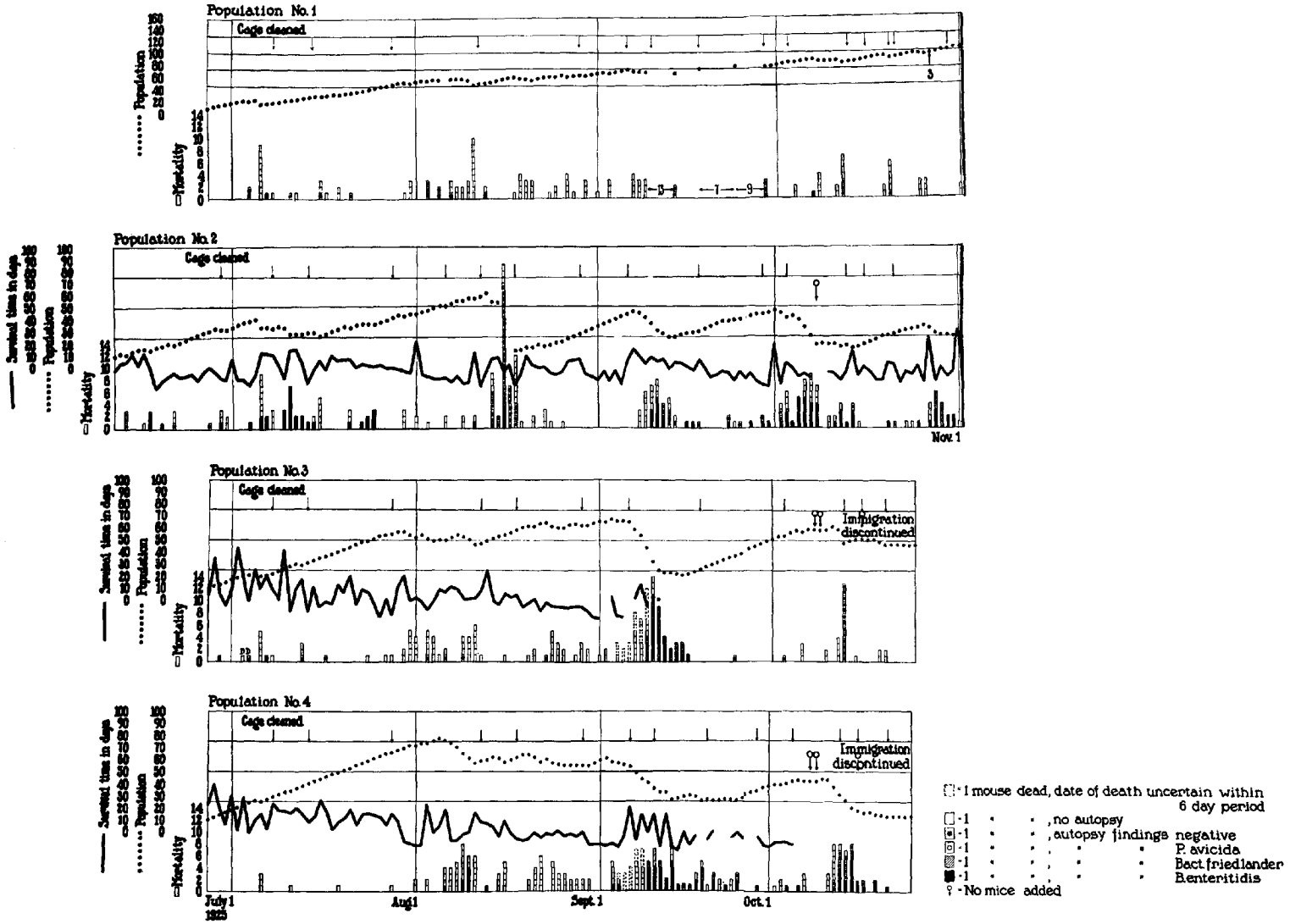
These events demonstrated three facts of epidemiological significance; first, that a "foreign" strain of a highly pathogenic bacterium, *P. avicida*, survived for 2 months in a previously unexposed population without producing obvious injurious effects; second, that after this period of apparent inactivity, the organism led to a severe epidemic fatal in 5 days to 47 mice or 77 per cent of the population; and third, that it failed to survive permanently in the community.

Comparison of Virulence of "Foreign" Strains and "Epidemic" Strains

A titration was made to determine whether the virulence of strains recovered at the height of the epidemic differed from that of the foreign strain originally introduced into the community.

Experiment 2.—Heart's blood cultures were taken from six mice which succumbed during the most severe phase of the epidemic, that is three on August 14, one on August 15, and two on August 16 (Text-fig. 1). On August 20, these six "epidemic" strains, together with the original "foreign" strain kept on blood agar and grown 18 hours in rabbit blood broth, were administered to test animals as follows: 0.5 cc. of each culture was given intranasally to two rabbits by instilling 0.25 cc. into each naris; each culture was also inoculated intraperitoneally in 18 mice in saline dilutions 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, 10^{-9}, 1 cc. of each dilution being given to three mice. The approximate numbers of bacilli administered were determined by plate method.

The results of these titrations are recorded in Table I. The number of organisms in each culture ranged between 2×10^8 and 5.1×10^8; hence effects of similar numbers of organisms of each strain could be compared. These effects were briefly as follows: the two rabbits receiving 2,300,000 bacilli of the original "foreign" strain died in 22.5 ± 1.5 hours, while the twelve receiving similar numbers of the six "epidemic" strains died in an average time of 22.7 ± 1.6 hours. All mice receiving the 10^{-2} dilution of the various cultures—2,000,000–5,100,000 organisms—were dead within 18 hours. The 3 mice receiving 23,000 bacteria of the foreign strain died in an average time of 18 hours, while 18 mice receiving 20,000



TEXT-FIG. 1. Spread of Pasteurella in mouse populations. For continuation see Papers II and III.

TABLE I

Comparison of Virulence of "Foreign" and "Epidemic" Cultures of Pasteurella avicida: Strain "Pa"

Culture	Mice												Rabbits	
	Dilution 10^{-2} No. organisms	Survival time Hours	Dilution 10^{-4} No. organisms	Survival time Hours	Dilution 10^{-6} No. organisms	Survival time Hours	Dilution 10^{-7} No. organisms	Survival time Hours	Dilution 10^{-8} No. organisms	Survival time Hours	Dilution 10^{-9} No. organisms	Survival time Hours	No. organisms	Survival time Hours
Stock "Pa"	2,300,000	−18, −18, −18	23,000	−18, −18, −18	230	20, 20, 21	23	39, S, S	3	S, S, S	0	S, S, S	115,000,000	24, 21
10/III/14	2,000,000	−18, −18, −18	20,000	−18, −18, −18	200	0, 21, 23	20	21, 25, 68	2	24, S, S	0	S, S, S	100,000,000	26, 21
5/IV/14	5,000,000	−18, −18, −18	50,000	−18, −18, 22	500	21, 22, 39	50	39, 39, 39	5	S, S, S	0	S, S, S	250,000,000	25, 21
4/V/14	4,000,000	−18, −18, −18	40,000	−18, −18, 20	400	20, 20, 23	40	20, S, S	4	S, S, S	0	S, S, S	200,000,000	24, 21
7/VI/15	5,100,000	−18, −18, −18	51,000	−18, −18, −18	510	21, 39, 68	51	18, 18, 24	5	23, 39, S	0	S, S, S	255,000,000	20, 23
1/VI/16	2,900,000	−18, −18, −18	29,000	−18, 20, 22	290	0, 39, 45	29	39, S, S	3	S, S, S	0	S, S, S	145,000,000	23, 21
3/V/16	3,100,000	−18, −18, −18	31,000	−18, −18, 20	310	−18, 20, 45	31	S, S, S	3	S, S, S	0	S, S, S	155,000,000	23, 25

0 = No animal injected.

S = Alive and well 30 days after injection.

to 51,000 bacilli of the "epidemic" strain died in an average time of 18.7 hours. The average time of death of the mice given 230 organisms of the foreign strain was 20 hours; while the mice given 200 to 510 organisms of the "epidemic" strains succumbed in about 30 hours; a dose of 23 organisms of the foreign strain resulted in 1 death at 39 hours and 2 survivors; similar small doses of the "epidemic" strains led to death in about 33 hours, plus 50 per cent survivors. Few of the 10^{-8} dilution animals and none of those receiving the 10^{-9} dilution died.

According to this titration, the pathogenicity or virulences of the original "foreign" strain and of the 6 "epidemic" strains were similar. It must be noted, however, that the titration was not performed under the best conditions because the rabbit inoculations involved the use of a foreign host and the mouse titrations the use of an unnatural portal of entry. Such a departure from natural methods, in which the native host and normal portal of entry are employed, detracts from the value of the test and furnishes at most presumptive evidence that the highly virulent "Pa" strain of *P. avicida*, gaining access to the mouse community, surviving and spreading with no apparent damage, finally was able to give rise to an epidemic outbreak of great severity without any demonstrable alteration in its disease-producing power.

COMMENT

These observations on Pasteurella infections in mice indicate that the primary response of average unexposed populations to foreign strains of the organisms depends on inherent pathogenicity, or virulence, of the microbes, that the inherent pathogenicity of the bacilli is relatively stable for mice, and that the strain with high killing power possesses low vegetative capacity.

SUMMARY

1. Three strains of Pasteurella native to rabbits, introduced into mouse communities failed to spread or survive.

2. One strain of fowl Pasteurella, so introduced, survived for 2 months without causing death, after which, it gave rise to a 5 day epidemic, fatal to 77 per cent of the population. Subsequently, no further deaths from this source occurred and the strain died out completely.

3. At the height of the epidemic, caused by the fowl strain of Pasteurella, 6 cultures were obtained which, on direct inoculation,

proved to have a degree of virulence for mice and rabbits equal among themselves and the same as the strain originally employed.

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