# AN OUTBREAK OF MOUSE TYPHOID AND ITS ATTEMPTED CONTROL BY VACCINATION.

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In this paper will be described briefly an epidemic of so called mouse typhoid which prevailed with fluctuations for approximately 2½ years in a mouse breeding station maintained at The Rockefeller Institute.

The original stock of about 3,000 mice of all ages was procured by purchase and transported *en masse* to The Rockefeller Institute. The stock was especially valuable because it yielded a relatively high percentage of mice developing so called spontaneous cancers, located in the mammary glands chiefly, but also in other organs. It is known that the original stock, from which that purchased by The Rockefeller Institute was derived, had previously suffered losses from mouse typhoid. Moreover, to the stock as purchased, there were added from time to time from the outside a mouse showing a spontaneous tumor or other small accessions of healthy stock.

The transfer of the mouse stock took place in April, 1918. Nothing especially noteworthy in the fatalities arose to arrest attention until about the middle of September of that year, when an unusual number of deaths among the stock occurred.[1] From this time on, until what may be called the termination of the epidemic period, some 2½ years later, the number of deaths may be regarded as having been abnormally high, although in intervals between the wave-crests, the death rate returned to a level as low as that usually regarded as normal.

It was customary to record all deaths occurring in the stock mice and at the beginning of each month to take a census of the population

[1] The summer death rate immediately preceding the September rise was slightly higher than usual. But as during the warm summer period previously a rise had been observed, this increase was not considered significant.

which fluctuated in number roughly between 2,500 and 4,000 individuals. The numbers as given by the census varied according to the matings and to the demands made on the stock for purposes of investigation.

It was the custom also to make a gross postmortem examination of every mouse found dead. When the deaths became so frequent as to excite apprehension of an impending epidemic, bacteriological studies were made. Cultures of the spleen and liver yielded a bacillus which was identified as belonging to the group of mouse typhoid bacilli. During the course of the bacteriological studies about a dozen strains conforming to the cultural characteristics of the group were isolated. Of this number two strains were retained in culture and transplanted regularly and thus kept alive. But it was not until about a year after the epidemic began that the two strains were subjected to immunological study by Dr. Amoss.

The two strains proved to be identical in cultural and immunological reactions, and there was no reason to suppose that the first epidemic was induced by more than this one strain of a bacillus belonging to the enteritidis group. As this strain was utilized by Dr. Amoss in certain of his experiments on the production of artificial epidemics among mice,[2] its more precise biological description will be found in his papers.[3] For sake of convenience the strain has been designated Mouse Typhoid I.

Before proceeding to the more detailed account of the epidemic among the stock of cancer mice, as they were called, a brief statement regarding the conditions under which mice are propagated at The Rockefeller Institute may be of interest.

Two distinct breeding rooms or stations for mice are maintained, with separate caretakers who do not mingle. One station houses the cancer stock and the other the normal stock. The latter has been inbred for the past 2 years, no outside accessions having been made during this period, and is employed in the general investigative work of the Institute. The completeness with which separation between the two stations has been effected is indicated by the fact that in the long period during which deaths—many or few—from mouse

[2] Amoss, H. L., *J. Exp. Med.*, 1922, xxxvi, 25.

[3] Amoss, H. L., and Haselbauer, P. P., *J. Exp. Med.*, 1922, xxxvi, 107.

typhoid were taking place among the cancer stock there were only four deaths among the normal stock apparently from this cause. The population of the normal stock was kept approximately at from 2,500 to 3,000 individuals.

*The Epidemic.*

The course of the epidemic outbreak can best be followed by observing the graphic curve which is reproduced in two sections which have been subdivided into segments (Text-fig. 1).

As the first segment (No. 1) of the curve indicates, the death rate among the mice rose in the 2nd week of September and actually became about twice the usual weekly rate. From this time on the records were kept more accurately than before and they show a gradual rise in the number of deaths up to the 1st week in November, when the rate remained constant for 10 days, increased during the 3rd week, and began to decline abruptly.

The curve indicating actual number of deaths forms a plateau extending over a period of 2 weeks during which there was a slight depression. The decline was slightly more irregular than the rise.

The course of the epidemic is perhaps better described by the attack rate per 1,000 of population, which rose sharply until November 19 and declined as rapidly as it had risen.

The return to what is regarded as the usual death rate came about January 18, 1919. The outbreak lasted about 140 days; the peak was reached at about the middle of this period, or the 80th day (November 19), so that the curve representing the rate per 1,000 is fairly symmetrical with a slight lag in the decline.

From a consideration of the character of the curve during December, there is a suggestion of the occurrence of another small wave which might have begun somewhere between December 15 and 20 and reached its peak on December 29. The interval between the peaks is about 15 days. Such a supposition is strengthened by the occurrence of a similar wave in March (shown in Segment 2) which endured for 15 days. It is not improbable that the total outbreak is really a series of six overlapping epidemic waves, each lasting about 15 days. Unfortunately no spot map was kept recording the distribution of the deaths in the breeding station. In general the epi-

TEXT-FIG. 1. Segment 1. Deaths from all causes among the cancer breeding stock by 5 day periods from September 5, 1918, to January 18, 1919.

Segment 2. Deaths from all causes by 5 day periods from January 19 to April 30, 1919.

Segment 3. Deaths from all causes by 5 day periods from May 1 to September 30, 1919.

Segment 4. Deaths from all causes by 5 day periods from October 1, 1919, to February 29, 1920.

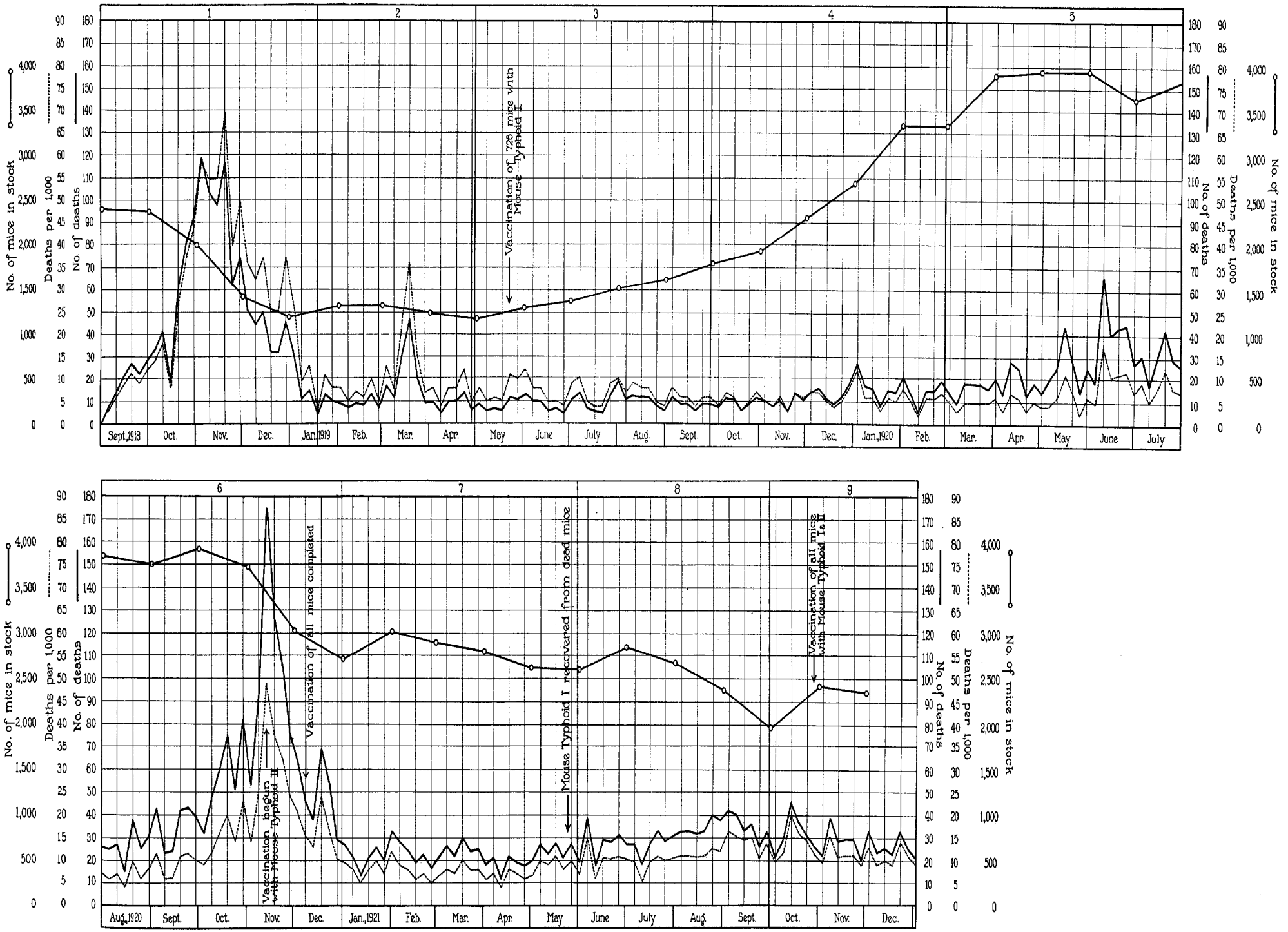
Segment 5. Deaths from all causes by 5 day periods from March 1 to July 31, 1920.

Segment 6. Deaths from all causes by 5 day periods from August 1 to December 31, 1920.

Segment 7. Deaths from all causes by 5 day periods from January 1 to May 31, 1921.

Segment 8. Deaths from all causes by 5 day periods from June 1 to September 30, 1921.

Segment 9. Deaths from all causes by 5 day periods from October 1 to December 31, 1921.




TEXT-FIG. 1. ----------Deaths per 1,000 of mouse population by 5 day periods. ——— Actual number of deaths in mouse population by 5 day periods. ——— Total number of mice in stock according to the monthly census.

demic raged on the shelves where the older mice were kept. For example, among 1,465 older mice the mortality was 82 per cent, whereas among 1,034 new mice, most of which were weaned before the onset of the epidemic, the mortality was 43 per cent.

The severity of this first, or as it may be called November epidemic wave, is shown by the number of deaths presumably in all instances from mouse typhoid. This number as calculated for the period from September 10 to January 28 inclusive is 1,351 mice, or a little more than one-half of the population of the breeding station. During December the deaths occurred mainly in boxes which had already been attacked. Instances of spread to new boxes were frequent.

It is quite certain that this large number of deaths was brought about by the mouse typhoid infection, for while not every dead mouse was studied bacteriologically, yet all were submitted to autopsy and the usually obvious signs of the disease detected.

Along with the epidemic there occurred as a concomitant effect a large reduction in the birth rate so that the number of mice diminished through death was not augmented by births. Hence during the next period of January, February, and 1st week in March, as shown by the second segment (No. 2) the death rate, while still high as compared with the pre-epidemic period, kept quite uniform. However, during the 1st week of March an upward tendency is evident, which at the beginning of the 2nd week is converted into a steady rise culminating in a peak lower than the November peak, enduring a shorter time, and falling more sharply, as the average death rate is again reached in the 1st week of April.

This second, or March epidemic wave is to be considered in the light of two classes, as it were, of the mouse population; namely, the old population which had passed through the November epidemic, and the accessions through new births. The total population had been profoundly reduced by the November epidemic and not yet restored by new births. The March wave was of short duration (15 to 20 days) and was approximately half as severe as that of November. As already stated, the November outbreak may be looked upon as a series of waves following closely one another, whereas the March epidemic represented a single wave.

A period of relative quiescence now set in, as shown by Segment 3. On May 17 the total mouse population was about 1,250 individuals. During the next 4 weeks, half of the mice were given (vaccinated) under the skin of the back a single injection of 0.2 cc. of a suspension (or about 600,000) of killed bacilli of the strain of mouse typhoid isolated during the epidemic. The vaccination produced no immediate effect on the death rate, although it is noteworthy that during a 4 weeks period of mid-May to mid-June, only two of the vaccinated mice died and in only one was the mouse typhoid bacillus found.

The carrying out of the vaccination would seem rather to have coincided with than to have been responsible for the quiescent period which extended from June, 1919, to about January, 1920, the level of which is shown in Segments 3 and 4. During this period the mouse population was rising steadily chiefly through new births. The new individuals were left with the old and no further vaccination was carried out in the breeding station. Although the general death rate had not fallen to that of the pre-epidemic period, yet the new equilibrium which had been established was regarded as satisfactory.

However, beginning in January, 1920, as the population continued to increase, a small rise in death rate also occurred as is shown in Segments 4 and 5. By May, 1920, the total population in the breeding station had reached approximately 4,000. As presaging of the next epidemic, events may be detected in the rise in number of deaths in April, May, and June, as indicated in Segment 5. Although the population had again become large, this mere numerical increase was followed with some anxiety.

The number of deaths per 1,000 continued generally high during July, August, and September. Beginning the 1st week in October, a further rise took place that with minor and perhaps unimportant fluctuations led into the sharp epidemic wave of November, 1920, which exceeded in actual number of deaths that of the first, or 1918 November wave, but the rate per 1,000 was only five-sevenths of that recorded in the 1918 outbreak. The course of this epidemic wave which was practically obliterated by January, 1921, is shown in Segment 6. The wave-like character of the curve is also present. Whereas the epidemic of 1918 seemed to be a summation of six waves, the 1920 outbreak consists of only four, each of which was longer and lower than in the former.

An analysis of this epidemic wave yields the following data. The total mouse population affected by this epidemic wave during its entire course was 4,282, of which 1,463 died. Hence the gross mortality was 34 per cent. Of the 4,282 mice in the total population, 220 were at least 18 months old and had thus passed through the previous severe epidemic wave, while 4,062 were new individuals not so severely exposed. Taking the two classes of the population separately, the old mice (18 months old at least),[4] and the new mice as above described, it may be stated that of 220 of the former, 130, or 60 per cent, succumbed in the November, 1920, epidemic wave, while of 4,062 of the latter, or new stock, only 1,333, or 32.8 per cent, fell victims. The effect of the epidemic on the birth rate was marked. During the extreme height and the following wave in November and December, very few births took place. By January 1, 1921, the total population had fallen to 2,731 and the sharp outbreak may be regarded as having come to an end. For the succeeding 5 months (January to May) the death rate remained at the average low level ordinarily observed in stocks of mice (Segment 7). The usual[1] slight increase in death rate was observed during the summer (Segment 8) and autumn (Segment 9).

There is practically no difference in the seasonal distribution of the two major outbreaks. Each was preceded by a slightly increased death rate during the summer months and began to gain headway in September, reaching the peak on November 19, 1918, and November 13, 1920, respectively. Since the death rate is calculated on a basis of 5 day periods, the difference of 6 days is probably within the limit of error. The entire duration of the 1918 outbreak was about 140 days and of the 1920 epidemic 125 days.

The bacteriology of the November, 1920, outbreak has particular importance. Practically all the dead mice were examined post-mortem, and cultures of the mouse typhoid bacillus were obtained by Mr. Sturm from approximately 75 per cent of the examined animals. Of the many cultures thus obtained, two strains were retained and eventually turned over to Dr. Amoss for use in his experiments.

[4] The average longevity of mice in non-infected stock is about 2 years. Old age must be considered as contributory to a high death rate in such a group.

The immunological study by Dr. Amoss of these cultures showed that they differed from the original cultures isolated in the 1918 epidemic. A full description of this bacillus will be found in another paper of this series.[3]

The fact that the two strains of the paratyphoid-enteritidis group, differing immunologically from each other and both potentially capable of setting up severe epidemics among mice, were responsible for the epidemics separated from each other by 2 years, comes to have a special interest and may possess a particular significance in view of the vaccinations carried out in the period between the two epidemics. For superficially, at least, it appears that the inoculation of the killed cultures of the first bacillus shunted, as it were, that particular organism out of action while leaving the recruited population, both old and new, and the old even more than the new, subject to a second variety of the mouse typhoid bacillus. If this is at all a true statement of what has taken place in the second November epidemic, then the vaccination of part of the surviving population in May and June, 1919, was sufficient to protect the entire population from infection with the first variety of the mouse typhoid bacillus. This last point is obviously one that is open to experimental inquiry as is also the collateral point whether cross-immunity reactions occur between the two varieties of the *Bacillus enteritidis* with which we have been dealing.

No further outbreak of mouse typhoid of an epidemic character has taken place in the cancer breeding station since January, 1921. Just as a large part of the surviving mice was vaccinated after the November, 1918, epidemic wave, so vaccination of all the survivors was carried out at the height of the November, 1920, epidemic wave. The vaccine employed in 1920 was identical with that employed in 1919, and as the two varieties of inciting bacilli differ immunologically, it may be regarded as at least questionable whether the second inoculations have had anything to do with the relative quiescence of the mouse typhoid infection in the breeding station following the last November epidemic.

A slight increase in the death rate occurred during the summer of 1921, and since Mouse Typhoid I had been recovered from some of the mice dying in May the entire population was vaccinated with Mouse Typhoid I and II in October.

*Carriers.*

No extensive study was made of carriers. In January, 1922, ten mice which had passed through the second epidemic and ten mice born of these were killed and cultured. The cultures were negative for Mouse Typhoid I and Mouse Typhoid II, except for those from one mouse in the former group. In this mouse Mouse Typhoid I was recovered from the cecum only. Cultures from the small intestine were negative for Mouse Typhoid I and Mouse Typhoid II.