THE ADVANCEMENT OF EPIDEMIOLOGY THROUGH EXPERIMENT *

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II

In September, 1918, a spontaneous epidemic of mouse typhoid appeared in a breeding population of 2,500 mice at the Rockefeller Institute and continued, with intervals of low death rate, for two and a half years, during which time, through natural increase, the total numbers fluctuated between 1,500 and 4,000. The epidemic was followed closely, a mortality chart was kept, dead animals were autopsied systematically and occasional bacteriological examinations were made. The epidemic finally ended, the cessation being hastened probably by systematic inoculation with killed cultures of the mouse-typhoid bacilli isolated from succumbing animals. It was the timely occurrence of this epidemic which afforded the direct impulse to the experimental studies, still being pursued, the outcome of which will now be presented. It is, however, proper to record that our minds were receptive, ready, as one might say, to take advantage of this chance offering to study epidemics thus directly in animals, as the result of experiences with recent epidemic diseases in man.

When the decision was made to attempt the experimental reproduction of epidemics of mouse typhoid, Amoss and I were not aware that a similar undertaking was well under way in England. Topley, whose investigations have been so fruitful, had already made a partial report in his Gulstonian Lectures which had just appeared in print. Our plan differs somewhat in detail from the one Topley followed, for while Topley mingles infected and healthy mice introduced at intervals in large cages, Amoss and I set up a kind of mouse village in order to follow as closely as possible the spread of disease from cage to cage. In both instances the epidemics were started by feeding a small number of mice pathogenic cultures of a mouse-typhoid bacillus (enteriditis-paratyphoid group of bacilli). After a few days' interval or incubation a part of the fed mice developed and later succumbed to infection. To this source of disease healthy mice were exposed, either immediately, as in Topley's or

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mediately, as in Amoss' and my experiments. We depended on the transfer of bacilli taking place through contamination of the hands of the attendant who fed the mice and cleaned the cages very much as we suppose happens in spontaneous epidemics; it is interesting to note that no degree of mechanical or chemical disinfection sufficed to render the attendant's hands free of the bacilli.

In the half dozen years which have elapsed since the investigations have been under way there has been a good deal of parallelism between the experiments carried out by Topley, and Amoss, Webster, Pritchett and myself, and latterly by Neufield and Lange, the actual results of which have been remarkably harmonious although the interpretations have not always coincided. There have also been differences in kind of experiment, according to the progress made in London or New York, and to the deductions separately based on the results obtained, as inevitably happens among experimenters and is conditioned by the particular imaginative outlook of one or another group of workers. It is a pleasure to record that between the English and American investigators the best of feeling has always prevailed, and through correspondence and actual visits the progress of events has been closely followed, and thus far without the slightest conflict and with common advantage all around.

It became immediately evident that it was possible to start continuing epidemics of typhoid among a healthy mouse-village population, from which it was concluded that a wide, if not uniform, distribution of the bacilli was quickly effected by the attendant, leading to infection and death of some but not all the mice exposed. At the outset the number and distribution of deaths in the separate cages simulate the sporadic happenings noted often to precede the real epidemic outbreaks. This preëpidemic variety of the disease was later, as will appear, converted into a true epidemic prevalence, in which groups of mice sickened and died according to an order, suggesting, if only roughly at first, the periods or following waves of the spontaneous mouse epidemics, or epidemics as observed in man. The explanation of these events, observed by Topley in his single-cage experiments and by us in the mouse village, was not at first wholly perceived. Topley ascribed them chiefly to rise and fall of bacillary infectivity, Amoss and I to fluctuations of virulence plus dosage of the bacilli. Later discrepancies could be accounted for by Webster, who determined the interaction of three quite constant factors, namely, level virulence of the bacilli, quality of resistance of the host or exposed mice, and quantity of bacilli ingested or dosage.

To enable you to follow the succession closely, let me state that in the Topley's experiments once infection was started fresh increments of healthy mice were placed in the cage at intervals. In Amoss' experiments once a kind of equilibrium or stationary period of disease arose in the village, cages containing fresh, healthy mice were brought into contiguity with the previously exposed animals. In order to be brief, I will give you the bare results of a long series of observations of successive waves of mouse typhoid followed by recessions, thus induced, in which not only the new animals but the old or already exposed ones also were affected.

In three separate experimental attempts to induce outbreaks of mouse typhoid resembling the epidemic spread witnessed in breeding stocks and spoken of as arising spontaneously, the type of disease induced was first that of the sporadic prevalence, as shown by the number of mice dying and the number of cages attacked. Once, however, this state of sporadic disease is started in a mouse population, all that appears necessary in order to convert the occasional deaths with low cage-attack rate into frequent deaths with high cage-attack rate, is to bring into contiguous relation with the infected population increments of not previously exposed healthy mice.

The sporadic prevalence is quickly over. The new mice having been introduced, the succeeding events proceed in an orderly fashion. After an interval of about five days the latter, or new mice, begin to die, the number of deaths and the proportion of cages attacked rising day by day. During the first period in which the new mice fall victims to the disease the old mice do not show an increased death rate. *But from the tenth to the twentieth day after the addition of the new mice, and hence the fifth to the fifteenth day following their deaths, the old mice are drawn into the wave of fatality, with the result that ultimately they suffer a mortality equal to or even greater than the new ones.*

The epidemics thus inaugurated, as indicated by the deaths, usually diminish or even disappear before all, sometimes after only a small part of the exposed mice have been destroyed. A tendency shown by all the experiments is for a state of equilibrium between the surviving mice and the infecting bacillus to be effected, this state continuing until fresh infectible material is provided, when there arise undulations or epidemic waves of deaths remarkably uniform in respect to the successive replacements of fresh mice. The undulations cease gradually, the late fatalities resulting not from delayed infection so much as from protracted illness and long sur-

vival. Bacillus carriers arise in the course of the epidemics and show, through possession of agglutinins, the existence of a partially immune state. It is through these carriers that the disease is propagated, in spite of which, as the events prove, the partially immune individuals are not themselves adequately safeguarded, since, as we have seen, the older mice which have weathered one or more epidemic storms ultimately sicken and succumb. Here another factor operates, and this all-important one in overcoming natural and acquired resistance is the factor of quantity or dosage of the bacilli.

Topley, as already pointed out, and his predecessor, Danysz, considered fluctuation in virulence as the factor which chiefly determined the waves of epidemic prevalence of mouse typhoid, and Flexner and Amoss regarded the virulence plus dosage as the deciding events. But it was the precise studies of Webster and Pritchett, on the constitution of the bacilli on the one hand and the reaction of the host on the other, which have yielded the constants with which to measure the actual happenings observed.

We are, as one might say, in sore need of the simplification of the phenomena of the epidemic, which happily an accurate study of the living materials and other effective agencies seem capable of supplying. We need to measure not only bacilli and host, but such other factors as diet and season, if we wish to bring animal and human epidemic occurrences into harmonic relationship. Undoubtedly, certain of these extrinsic influences have been included in the term "telluric", as used by the older epidemiologists. Fortunately we may subject certain of the "influences" to experimental verification in order to introduce precision into our calculations, in opposition to such vague notions as "epidemic constitution". It would seem not inappropriate to introduce at this point of our discourse the significant words with which the young army surgeon, Helmholtz, as he was then, closes the introduction to the treatise giving to the world the mathematic physical demonstration of the conservation of energy, based in part, as you may recall, on physiologic studies carried out on voluntary muscle. He says:

"Theoretical natural science, therefore, if she does not rest contented with half views of things, must bring her notions into harmony with the expressed requirements as to the nature of simple forces, and with the consequences which flow from them. Her vocation will be ended as soon as the reduction of natural phenomena to simple forces is complete, and the proof given that this is the only reduction of which the phenomena are capable."

With this ultimate purpose in mind we may now proceed to an analysis of the phenomena of mouse typhoid as experimentally induced in relation to such factors as constitution of bacilli, response of host and influence of dosage, diet and season, as determined by the precise studies of Webster and Pritchett upon homogeneous stocks of mice.

Bacilli were administered *per os* by means of a simple mechanical device, which delivered a fixed quantity, approximately 4,000,000 organisms, into the stomach. Five strains of enteriditis paratyphoid bacilli were tested on about 1,400 mice. Stools and blood were studied by culture, and blood was tested for agglutinins. Three groups of mice were distinguished. In the first group the bacilli disappear at once or after a brief period from the stools, and the blood is persistently negative for bacilli and agglutinins; the twenty to thirty per cent of mice composing this group continue well during the eight-week period of observation. In the second group the bacilli are passed for an irregular period; small numbers invade the blood and agglutinins are present and strong three weeks after the inoculation. The five and ten per cent of the mice composing this group sicken but recover. The members of the third group pass large numbers of bacilli, many are cultured from the blood during life, and the seventy to eighty per cent of mice composing it sicken after five days' incubation and sooner or later die. The five bacillary strains differed among themselves in degree of virulence, but each one remained constant in its pathogenic action upon mice and was unaffected by animal passages. It could be shown, therefore, that the epidemic outbreak of greater or less severity of mouse typhoid is determined in large measure by inherent degree of bacillary virulence. An originally highly pathogenic strain is more destructive than an originally less active one, and antigenic identity is no measure of ineffective equivalence. In other words, the occurrence of two strains of the bacilli pathogenically different and antigenically identical does not indicate transmutation of one into the other. When suitable tests are carried out by intraperitoneal inoculation the same general rule regarding virulence fixity of strains are found to apply.

In order to determine the limits of the host response the effect of varying dosage has first to be ascertained. When fixed quantities of cultures of given strains of mouse-typhoid bacilli are introduced into the stomach of mice of selected weight, and of the same lots as regards parentage and food, consistent results, as shown by the per-

centages of those surviving and succumbing, are obtainable. When massive doses of the bacilli are given the deaths fluctuate between seventy and eighty per cent; when critical doses are given the percentages vary from forty to fifty. When infection is permitted to take place by contact of inoculated with uninoculated mice the end result is determined by the relative numbers of each kind in the combination. Thus when five inoculated mice are placed with one uninoculated animal the percentage mortality of the latter corresponds with that of the purposely inoculated, while when the ratio of inoculated to uninoculated is one to one the effect of the contact is very small.

These findings bring out two important points, namely, that the susceptibility of a mouse population to mouse-typhoid infection is a relative or graded property; and that the dosage, as determined by the quantitative and spatial distribution of the bacilli, is a definite factor or constant, as shown by the mortality curve. A significant corollary to the findings is that irrespective of reasonable dosage and of strain of bacilli, from twenty to thirty per cent of mice ordinarily resist infection.

The statement just made regarding resistance indicates that using sufficient numbers of mice, the quality or degree of host susceptibility or resistance, according to the angle from which the results are viewed, may be found also to be a constant. Thus 540 mice of the same age and weight, bred at the Rockefeller Institute, were given *per os* a standard dose of culture of a mouse-typhoid bacillus. The mortality curves of the several tests were remarkably uniform. There were, it is true, minor variations which later, and as will appear, could be correlated with seasonal changes. The figures yielded by this larger number of mice were employed to construct a so-called *standard control curve,* with which other curves could be profitably compared. For example, it was found that when mice were drawn from other sources and inoculated in an identical manner the curve differed from the standard one. Thus, mice procured from a Pennsylvania source yielded a curve lower than the standard, and mice from a New Jersey source, a curve exactly agreeing with the standard.

The constancy of response among groups or stocks of mice to given strains and doses of the bacilli, suggested to Webster that after all the determining qualities residing within the animals might well be not of specific, so much as of nonspecific nature, and something which comes into play, no matter what the nature of the poisonous agency may have been. With this idea in mind, he administered

bichlorid of mercury also by stomach tube to groups of mice. It happened that with a dosage of 0.0033 gm. the mortality curve exactly parallels the standard curve. With larger and smaller doses of the drug, curves are obtained reproducing those yielded by culture strains of higher and lower degrees of virulence.

Up to this time the host material consisted of mice of common stocks, but not of pure-line origin. That heredity may affect the degree of susceptibility could be assumed. Fortunately, material was available to test this point directly, as was done by Pritchett. Two sets of experiments, extending over two years and involving five separate strains of mice and five thousand individuals, were made. The strain consisted of three pure-line lots yielded by brother-sister matings and two homogeneous lots obtained by close inbreeding. During the first year five hundred and during the second year from three hundred and six hundred mice of each strain were inoculated *per os* and closely followed. The result of this laborious study leaves no doubt that definite differences in susceptibility to typhoid exist among mouse stocks and that hereditary factors play an essential part in determining variations.

Having now ascertained that the quality of the host plays a leading or decisive part in mouse-typhoid infection, it would seem to follow that the mice resisting bacterial infection should be the ones responding least to mercury bichlorid poisoning, as indeed was found by Webster to be the case. Moreover, he also found that when mice surviving ordinary lethal doses of mouse-typhoid bacilli are inbred for several generations, the offspring become progressively more resistant to bacilli and chemicals than the corresponding unselected mouse material.

With these data before us, we may turn for a brief consideration of certain of Topley's findings for which he gave no inadequate explanation. It will be recalled that he assembled mice in large cages to which fresh animals were added at intervals. He found that when the additions were made in the period of decline of the epidemic the fresh mice were more likely to survive than when they were added at the ascent of the wave; also when the additions were made daily the deaths did not occur regularly, but came in waves nevertheless. Topley ascribed these happenings mainly to supposed fluctuation in virulence of the bacilli. We now know, thanks to Webster's accurate studies, that the bacilli do not so fluctuate, and we have also learned that host susceptibility and dosage of the bacilli largely affect infection and death. The probability, therefore, is that the

wavelike movements, observed originally by Danysz and later by Topley and ourselves, in the course of mouse-typhoid epidemics are determined by the quantitative distribution or concentration of the bacilli acting on mouse populations previously unexposed. In all instances first the former and next the latter are affected, according to the measure of original susceptibilty and subsequent available quantity of bacilli. That this is the usual series of events was shown by Pritchett in a conclusive way. She devised means of estimating at two or three days' intervals the number of mouse-typhoid bacilli in a cage containing an infected population, to which daily additions of two healthy mice were made. The tests carried over a six-month period showed definitely that the mortality rate rose regularly six to eight days after a large increase took place in the number of bacilli in the cage.

Dosage of bacilli, therefore, in mouse typhoid—just as concentration in certain infectious diseases in man—is found to be a significant factor in initiating epidemics. It is well known that in order to diminish greatly, or even to abolish altogether malaria and yellow fever, we need not eliminate all the mosquito sectors; what is aimed at is to reduce the number to an established effective index, when the diseases disappear. When certain bacterial infections rage, streptococcus sore throat, for example, the number of carriers of hemolytic streptococci is large; as the malady abates, the number of carriers shrinks, and so with epidemic meningitis and other diseases. It is desirable that the fact be generally appreciated that in quantity of bacteria we have something which can break through the resistance barrier, be it natural or acquired. The persons vaccinated against smallpox or against typhoid fever, adequately protected under ordinary conditions of incidence of those diseases, may still be open to attack when the disease becomes rampantly prevalent. The protective immunity mechanism is a measurable quantity, and may be superseded. When the quantity or dosage, so to speak, of the microbic assailant is large, and the grade of virulence high, even the extraordinary defenses of the body may prove insufficient.

We have now reviewed the inherent factors, microbe and host, as we may call them, as they react on each other, in the course of those happenings through which epidemics are generated. It will, I think, be admitted that the knowledge gained by the experimental method of study of the phenomena displayed by mouse typhoid is enlightening. In how far the data secured can be applied to other epidemic diseases and to hosts other than mice, has still to be determined.

We know already, thanks largely to Webster's investigation of *Bacterium lepisepticum* infection in rabbits, that they are applicable to them. But we have still to consider certain external or environmental influences which affect the course of mouse typhoid and doubtless of other diseases as well. I shall deal, at this time, especially with the influence of food and of season on the course of mouse-typhoid infection.

That food should play a part in altering the course of an epidemic disease might perhaps be inferred from the profound effects produced by dietary deficiencies as expressed in beriberi, rickets, scurvy and possibly in pellagra. We shall deal, however, in this instance with no such extreme departures from the ordinary in diet. Indeed, it has been found—and just this is the suprising thing—that a diet to all intents and purposes adequate and favorable may nevertheless affect a stock of mice injuriously in comparison with another diet more ideally, as it seems, adjusted to their requirements.

Webster and Pritchett's experiments on food effects were simple in design. Two sets of mice were employed. One set was reared on the regular Rockefeller Institute ration in use for many years, and consisting of a daily feeding of baker's bread soaked in grade B pasteurized milk and warmed to 60° to 70°C., and supplemented by two weekly feedings of an oatmeal-buckwheat mixture and one weekly feeding of dog biscuit. The animals thrive and multiply on this diet. The diet with which this was compared was McCollum's normal one for rats and mice, consisting of wheat, 67.5 per cent; casein, 15 per cent; milk powder, 10 per cent; sodium chlorid, 1 per cent; calcium carbonate, 1.5 per cent; butter fat, 5 per cent. The mothers and the offspring, the latter being taken for experiment, were fed the McCollum mixture. Equal groups of mice fed on the two diets were given by stomach tube either about 4,000,000 mouse-typhoid bacilli or bichlorid of mercury. A very few of the mice were tested by intraperitoneal injection of 1 minimum lethal dose of botulinus toxin. The mice fed on the McCollum diet proved to be less susceptible to all three injurious agents. As the effect of the diet is directed against chemical as well as biologic poisons and in equal degree, we are justified in speaking of the defensive mechanism involved as nonspecific. This fact leads to the important conclusion that the factor which we so commonly invoke under the names of susceptibility or resistance is an innate quality of the host, brought into play under stress and strain of injury of widely various nature.

Seasonal variations of disease are, of course, very well known.

Even when we exclude those diseases which depend upon insect vectors whose prevalence is determined by season, we still meet with examples which display strong seasonal fluctuations. The influence of season was tested on 5,000 mice by Pritchett in two sets of experiments covering the twenty-four month period from 1923 to 1925. A given number of mice, consisting of three pure-line and two homogenous strains, was inoculated by stomach tube every month with about 5,000,000 mouse-typhoid bacilli of fixed virulence. Each test was run for eight weeks, the deaths being recorded daily and routine bacteriologic examinations being carried out. Not only were seasonal variations displayed, but they were remarkably uniform over the two twelve-month periods; yet certain, if slight exceptions arose. As the mouse material was by heredity of heterogeneous nature, certain differences in response to infection resulted from that circumstance. And yet it may be stated that the course of events was uniformly such that the mortality curves tended to arrange themselves into three series, namely, curves indicating: (*a*) High spring mortality; (*b*) low summer mortality; (*c*) intermediate fall and winter mortality. Expressed in terms of deaths, we may say that the spring rate is high, the summer rate low and the fall and winter rate, while falling between the two may in the early fall rise sharply and even approximate the spring level. Since, however, seasons, year by year, are only roughly comparable and vary in temperature, sunlight and other conditions week by week or month by month, a certain amount of fluctuation in the curves is to be expected, provided that it is affected, as it appears to be, by meteorologic occurrences.

It would be desirable, in view of recent discoveries of the profound effect of light in protecting the animal organism from rickets through its influence on the calcium metabolism, to study the part which it may play in epidemics. Indeed, the recent precise studies of Brown and Pearce on the effects of sunlight, especially on the normal as well as on the pathologic organism, already point to important correlations. They have secured data from 400 normal rabbits, observed month by month over a period of three years, and a like number of rabbits inoculated with a malignant tumor and with syphilis, similarly studied. They have found first that, contrary, to current belief, no fixed relation of size and weight attaches to the individual organs, but that the relationships are constantly changing in a more or less rhythmical manner. Hence what might be regarded as normal at one time would be regarded as abnormal at another time. The weights of the heart, liver, kidneys, and even the brain

in proportion to body weight, fluctuate in the course of a year as much as twenty to forty per cent; while the endocrine organs, lymph nodes and spleen show variations of fifty to one hundred per cent. Constant readjustments of the relative balance among the various organs of the body take place, and this system of changes follows a regular course which may be correlated with the seasonal changes of the year. So far the meterologic condition which occupies the place of most importance is sunlight. Just as the organs increase and diminish in relative size, so it appears do the severity of the tumor and the manifestations of syphilis in the rabbit rise and fall according to the organic changes. From this it may be inferred that the seasonal variations affect functional activity in that they coincide with or induce alterations of the state of the animal body with which pathologic effects are related.

We have now arrived at a natural halting place in this discourse, from which to view the ground that has been covered. We may, I believe, discern a certain order and sequence of events in determining epidemic ocurrences in at least one infectious disease in animals. Not only may the origin of mouse-typhoid epidemic be accounted for by the presence of the offending bacillus, but the course pursued, is as it were, predictable on the basis of the constitution of microbe and host as affected by such modifying causes as dosage and particular environmental conditions. The preponderant role of dosage and fresh infectible host material is clearly indicated in prolonging the epidemic and explaining the periodic wavelike movements. That the spontaneous epidemics of mice are prolonged in an identical manner by new births and by the spatial and quantitative distribution of bacilli may be assumed as quite certain. Application of these findings to the study of epidemics of human diseases in which more precise observation shall take the place of actual experiment is the possibility clearly indicated. Perhaps in this manner will the next forward step be taken leading to the solving of riddles in epidemiology as related to man.

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