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THE ADVANCEMENT OF EPIDEMIOLOGY THROUGH EXPERIMENT *

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Within the quarter century just closing, the United States together with many other countries has been passing through four considerable epidemics of disease—of cerebrospinal meningitis, poliomyelitis, influenza, and epidemic encephalitis. Each of these plagues has, either in turn or contemporaneously, claimed many victims, and excepting perhaps epidemic influenza, none of them has definitely run its course. The ravages still continue, and it is indeed not to be predicted when they are to cease and the conditions prevailing before their appearance be resumed.

There is small wonder, therefore, that the study of epidemiology should have come again to the front. A sorely harassed world is awaiting anxiously, as it were, a satisfactory answer to the question of the nature and the source of these pestilences of mankind. The question is of course not a new one, and already not a few answers, none really adequate, have been made to it. But the state of the medical sciences at the present day would seem to give hope that knowledge not hitherto available may be secured from a fresh attempt at the solution of the biological problems involved.

We turn back to the father of medicine, as Hippocrates is admiringly and affectionately called, for the first recorded observations on the epidemic occurrences of disease. For it is true that the chapters on epidemics in his works consist of observations in which clinical course and states of the weather are considered in mutual relationship. Through this, then novel, way of viewing the onset and the symptoms of disease arose the doctrine of epidemic constitution, which has since played a major, if fitful part, in the history of epidemics. In developing this concept as applied either to climatic conditions of such marked type as to give a distinguishing character

* Extracts from the Wier Mitchell oration delivered at the College of Physicians, Philadelphia, and which was delivered in part, by Dr. Flexner at the School of Tropical Medicine of the University of Porto Rico.

to a period of time or to denote a fixed type of disease prevalent at a particular time, Hippocrates described actual occurrences which later writers translated into theories of the epidemic.

The further development of the concept of epidemic constitution as a controlling or determining agency in bringing about the rise and fall of disease is attributed to Ballonius and to Sydenham. The writings of the latter are more familiar to us and Sydenham's name is intimately associated in our minds with the doctrine. According to him the potent influences are engendered through occult and inexplicable changes in the atmosphere, and are of a mysterious, so-called "skiey" nature which determine the duration and devastation of the prevailing disease. The constitutions differ in different years, depending upon hidden changes in the bowels of the earth, through which the atmosphere becomes contaminated and the bodies of men predisposed and made subject to this or that disease. A particular disease continues during the influence of a certain constitution, which after a cycle of a few years gives ground and makes way for another.

All this may sound to us extremely mythical, and yet very recently the concept of constitution has been, indeed is being, reasserted in very respectable epidemioligical quarters, in order to explain the epidemic happenings of the past twenty-five years. The question has been put whether in this period just such a cyclic succession of diseases and of constitutions, having a world-wide distribution, has not been taking place. Now it may be said without any comment in advance, that it is not improbable that a doctrine so vital as that of epidemic constitution has shown itself to be, may after all, and in spite of certain vaguenesses and repellant features, still contain a morsel of precious truth, the detection and elucidation of which may be essential to a complete understanding of the nature of the processes—biological and physical—on which the occurrence of epidemics depends.

In Sydenham's day, the sciences of statistics was in its infancy in England and had not yet been born elsewhere. With its rise and with the study of the written records of epidemics, there arose a new outlook in epidemiology which may be called the statistical-geographical, and which culminated, as it were in the publication of Hirsch's great work between 1860 and 1864 on historical geographical pathology. As presaging coming events which ushered in the bacteriological era, there appeared within this period two remarkable papers dealing with the innate nature of infectious disease, and postulating with a certainty and completeness not before at-

tained the necessary or casual relation of microrganisms to the processes. I refer to Henle's epochal paper, "On miasms and contagia" of 1840 and the less celebrated one by Greisinger on "Typhus, typhoid, relapsing and malarial fevers," published between 1857 and 1864.

Hard on the heels of Hirsch's monumental work came the epochal discoveries of Pasteur and Koch, their pupils and successors, which quickly transformed the entire scientific and practical outlook on the infectious diseases. The extraordinary discoveries in bacteriology which now followed in almost bewildering rapidity of succession, raised strong hopes that the inner nature of the epidemic would be quickly revealed, a premature expectation which I need not tell this audience has not been entirely realized.

And yet the gains even within this field have not been small. I need merely to allude to the detection of the insect vectors of disease and the diminution brought about through the practical application of this knowledge to malaria, which in many places has been thus and for the first time brought under control, and to yellow fever which has in the same manner been almost eradicated. Furthermore, through an adequately, scientifically based system of water purification, typhoid fever has been made to vanish from many strongholds, and the menace to the West of cholera has been reduced to impotence. The discovery of the power protective inoculation has served still further to diminish the incidence of typhoid fever and allied diseases, and of diphtheria; and the detection of the healthy and other carriers of disease-producing germs has been rewarded by still further material reductions.

And yet we are so far from a complete understanding of the nature of infection in all its variegated and intrincate forms as to be in certain instances, in epidemic influenza for example, impotent to affect its essentially undeviating course. The masters of bacteriology early perceived that infection is a process more subtle than the resultant of a mere bringing together of, on the one hand pathogenic microbe, and on the other susceptible host; and that about those factors played a series of adjusted biological reactions which ever since and increasingly it has become the task of the bacteriologist to disentangle. Somewhere within this field once it is wholly won over to knowledge, lie the opportunity and the power to interfere with precision and certainty for the control of epidemic disease.

The discrepancy between the power of the microbe to strike and that of the host to defend led to the formulation of certain counter

theories of the epidemic, in opposition to the purely bacteriological or etiological one coming into vogue. The counter theories still play a part in the history of epidemiology and, in one form or another, continue to be invoked, as has indeed just been done by Wolter in the case of the soil theory of von Pettenkofer, to account (I believe erroneously), in Russia especially, for postwar epidemics of typhus and other diseases which are, I think more satisfactorily explained on the basis of famine, louse, infestation, and like causes. This notion put forward by von Pettenkofer—postulates a certain prepared state, contamination or what not, of the soil as a forerunner and concomitant of epidemics, especially (although not exclusively) epidemics of typhoid fever and cholera. The theory has, with growing knowledge and improved communal sanitation, steadily lost ground and its revival seems inopportune and is not likely to bar real progress. On the other hand, Nägeli's diplastic theory, so-called, which assumes the coordination of the two microbes—one as we should now say, the specific, etiological incitant, visible, cultivable, and so forth; and another, hypothetical and probably ultramicroscopic—has received an unexpected ally recently in Hamer, the British epidemiologist, who sees in the class of filter-passing organisms the second essential factor in the partnership. Basing this view on de Schweint's discovery of the virus of hog cholera, Hamer constructs an imaginary pathology of influenza and other epidemic diseases, in which the filter-passer determines the kind of action which the specific, more obvious incitant, as, for example, the Pfeiffer bacillus, shall perform.

We may discern in these revivals—epidemic constitution, contamination of the soil, cooperative or symbiotic action—the serious difficulties under which present-day epidemiologists are contending in the effort to bring light and order into the discussion of the nature and origin of the epidemic. The statistician too is groping more or less in the dark. The science of statistics has grown amazingly since Sydenham's day, and it is applied to an ever-widening circle of happenings, and often doubtless with remarkable results. As applied to epidemiology, we are enabled to envisage the perturbation of epidemic disease as never before: We follow now its imminence, onset, rise and decline on a world-wide scale. By substituting, to use Greenwood's phrase, the herd for the individual, through personalization of disease phenomena, we have undoubtedly procured a body of data for analysis leading sometimes to useful or suggestive deduction. To mention one or two examples only, one thinks of Farr's generalization of the course of the epidemic in

cattle plague in England, and Brownlee's hypothesis of a life cycle of the measles or other incitant, still awaiting discovery, which controls the appearance, disappearance and reappearance of epidemic outbreaks of measles and possibly of other diseases.

The bacteriological era in epidemiology is distinguished from its predecessors particularly by reason of the concept of strict specificity, or the operation of a particular microorganismal agent in producing a definite disease. In this important respect, the periods before and after Pasteur and Koch are widely separated. The isolation of tubercle, typhoid and diphtheria bacilli, of trypanosomes and spirochetes, and a whole host of other microbes intimately associated with cases of infectious disease, and their clear biological distinction one from the other, led to the conviction of a particular parasite as the incitant of a particular disease. The strength of this belief in specificity is reflected in Koch's dictum that the parasite must always be found at the seat of disease and in the sick, but not in the healthy individual. In view of the frequency with which socalled "carriers" of pathogenic microbes are to be detected even among the healthy, this exclusive doctrine is no longer tenable, although it may not be affirmed that the newer discoveries in this field have severely shaken the concept of specificity. Rather it may be asserted that they have widened the outlook and led not only to the solution of riddles, otherwise unsolvable, in local outbreaks of infectious disease, but they have served to emphasize strongly the coordinate nature of infection and the interplay of forces as between microbe and host of previously unsuspected intricacy.

Up to this time the second partner, or host, had accorded him a subordinate, if not relatively an insignificant role. It became apparent, however, that not all individuals or groups equally exposed, equally responded to the exposure, and that with many epidemic diseases, sometimes racial, sometimes other characteristics seem to play determining parts. In regions in South America where yellow fever raged periodically, the dark or native population was observed to suffer less than the white immigrants, and the newly arrived among the latter oftener became victims than those already established or acclimated. Among animals, too, as in the notable instance of insusceptibility of Algerian sheep to anthrax infection, such distinctions have existed. Whatever, therefore, might be thought regarding the activity of the microbe agents of disease, it was obvious that the host material is not an homogeneous one, and that previous and prolonged exposure to an epidemic disease does tend to decrease danger of attack.

The detection of carriers and especially the greater refinements of the bacteriological technic in revealing microbes potentially pathogenic as forming groups rather than as composed of sharply cut species, raised the questions whether they also do not show more or less heterogeneity. A vast literature on this subject has already come into existence and to it there are made almost daily additions, the sum of which has tended greatly to modify the older views of the fixity of bacterial strains. I desire to place emphasis on the point that in the brief description which follows, we are dealing not with the transformation of species, in which the ground or original type, phylogenitically speaking, remains undisturbed.

In 1902, de Vries announced mutation among the higher plants and in 1906 N. Neisser and Massini described a mutating Bacillus coli which they termed "mutabilis". The twenty years intervening have witnessed the demarcation of large groups of related organims, such as compose the dysentery and paratyphoid bacilli, and the pneumococci, streptococci, and meningococci—to mention only outstanding examples—in which distinctions are based on cultural, pathogenic, and immunological differences. Instability of type among certain bacterial groups and transition between types have often been asserted, but the conclusive evidences for such conversions have been slow in being brought forward. On the other hand, it is logical to suppose that with wide fluctuation among microbes, affecting even the quality of virulence, the possibility is to be reckoned with of particular variants (mutants?) of enhanced powers of infectivity becoming dispersed and thus leading to epidemic outbreaks of disease.

The investigation of ordered variation or mutation among the bacteria was greatly advanced by de Kruif's observation of changes occurring in pure line strains of the bacillus of rabbit septicemia (Bacterium lepisepticum) and of Arkwright's observations on Bacilli typhosus, paratyphosus, enteritidis, and dysenteriae in 1921, through which the highly virulent S forms (growing in smooth colonies) become transformed into slightly virulent R forms (growing in rough colonies.* The two variants show immunological reactions more or less in common, but differ markedly in acid and saline agglutination points. The S strains tend in different degrees to give off R strains, while the latter doubtfully revert to S. In the case of the bacillus of rabbit septicemia, more closely studied perhaps than any other

* DeKruif used the letters D (diffusely growing in broth) and G (granular growing) to designate the two forms. But Arkwright's designations S and R have now been generally adopted.

bacterium in this respect, the change from R to S has not been detected. The S—R mutation of this bacillus has been shown by Webster to depend on the oxygen tension of the culture, and to be favored by free oxygen and inhibited by oxygen-absorbing substances (hemoglobin, etc.) in the medium. Reimer and Amoss, independently of each other, have recently promoted by special procedures the passage of pure-line strains of pneumococcus type I *S* into type I *R*, but have not been successful in bringing about the reverse transformation.

Nor is this all. The pneumococcus and streptococcus groups of bacteria are doubtless closely related, and the distinction between them has long been regarded as indefinite. If, therefore we are to accept recent perhaps inconclusive reports of Neufield and of Morgenroth, we must admit transformations within groups tending still further to obscure the dividing lines. Moreover, Jordan has even reported an instance of reversion of a pure-line paratyphoid R into S. Not impossibly not one variant only, but a series of S—R variants is given off by certain bacteria, some slightly, some fundamentally altered in relation to the parent strain, according to which degree the R—S reversion may or may not be accomplished. The studies in this fascinating field are progressing rapidly, and it will be well to withhold judgment and prediction until we are possessed of far fuller data than are at present available. I commend to those intending to embark on these somewhat troubled and tedious investigations, the utilization of pure-line strains only derived from single bacteria as procured by the Barber or an equivalent technic, and the employment of as precise and rigid bacteriological and immunological methods as may be possible.

To the epidemiologist this new knowledge and direction of research come with special force and significance. As his purpose is to account for the origin as well as for the movements of the epidemic, the life history of the microbes concerned becomes of high importance. Mutation or variation being now established as a not uncommon phenomenon, the question rises whether the change goes only in the direction of more to less, or may also proceed in the direction of less to greater virulence. *The fact should, therefore, not be loss sight of that up to now the changes observed have been of the nature of a degradation and not of an exaltation of pathogenicity*, and while it cannot of course be denied that the reverse process is capable of taking place, we must admit the lack of convincing evidence of its significant occurrence.

At this point I shall digress in order to present to you an instance

of rise in power of the virus of poliomyelitis which came to the attention of Dr. Harold L. Amoss and myself. Unfortunately, the conditions under which the rise, fall, and second rise in the activity of the virus took place were not such as to permit any clear insight into the precise factors involved, as for example, whether merely one alone or several virus strains collectively were involved. And yet the incident is not without suggestive value. But the circumstances under which it was noted are so onerous that the observation is not likely soon to be repeated.

Beginning almost with the appearance of epidemic poliomyelitis in the United States in 1907, experimental studies were carried out at the Rockefeller Institute. A strain of human virus was implanted in rhesus monkeys. It was at first of low virulence, but a few passages enhanced its activity for the animals, and soon maximal virulence was attained and maintained by systematic inoculations for a term of three years. Then for wholly unknown reasons, diminution set in and the virus returned approximately to the low level of the original human material. Samples of the nervous tissues of infected monkeys had been regularly preserved in glycerol at 4 C., and these samples were retested from time to time to determine activity. About six years after the virus had diminished in infectivity, a rise in power was noted, and a second high level was attained about equalling the first. The enhanced virus has remained at that level during the past five years. Briefly, this specimen of virus passed through several distinct phases of activity: low, high, low, and again high, the last stage while specimens of nervous tissue were preserved in glycerol for several years without being subjected to animal passage.

Certain epidemiologists, and notable Gottschlich, emphasizing the phylogenetic ascent of parasites from saprophytic bacteria, cite certain occurrences as indicating the transformation in nature of nonpathogenic into pathogenic organisms. Such a supposed instance is that of Uhlenhuth and Zuelzer who, by employing passages, believed that they accomplished the conversion of a saprophytic water spyral into a pathogenic variety. However, Neufield, an authority who favors the general point of view expressed, does not accept the interpretation given of this particular example. Gottschlich on the other hand, sees in the reappearance, after long cessation of cholera in 1817 and of plague in 1894 in India, the operation of natural causes transforming the corresponding saprophytic into parasitic incitants of these diseases, a conclusion which with present knowledge will be acceptable to few bacteriologists. Neufield among others

urges that pandemics of influenza arise, not through succesive distribution from one place to others of the highly virulent microbic incitant, but by reason of regional transformations of bacterial strains of low infectivity into strains of maximal virulence. That selected, more highly infective strains of pathogenic bacteria may be secured by animal passages is something pointed out by Pasteur and accomplished every day in the laboratory. But no experienced bacteriologist sees in this device the production of new or enhanced bacterial variants or species. The Germans, as do we, distinguish between mere separation or selection (Auslese) and modification (Umstimmung) by mutation or some other process of variation. Even as regards epidemic influenza, one cannot accept unqualifiedly the "local", as it may be termed, passage from low to high virulence of a microorganism, as accounting for the pandemics of that disease, seeing that outbursts follow lines of human travel, proceed in a geographical and chronological order, sweep past inaccessible communities, and show in general that characteristic to which the name "posting" has long since been applied.

Conscious of the inadequacy of the evidence adduced for the belief in microbic variation or mutation as accounting for the rise of epidemic, the protagonists of this view, as instanced by Neufield, hold that support for it is to be sought in the appearance of new diseases. If it is admitted that the parasitic organisms have evolved from saprophytic ancestors, then at some period in history, the specific infections which have endured, must have emerged and presented themselves for the first time. It is, of course, entirely possible that none of our laboratory procedures suffices to initiate the transmutations involved. We should, therefore, be on the lookout for the occurrence of new types of infectious disease, incited by microorganisms not previously known. No one would, of course, wish to reflect this quest, or deny the possibility of a successful issue. A pertinent question just now would be whether epidemic encephalitis may be regarded as a "new" disease. We are unfortunately in the weak position of not having discovered the incitant; and yet it is true that present-day internists and pathologists have had no previous experience of an epidemic disease of the nervous system corresponding to the multiform encephalitis. Even assuming that the incident were actually isolated and proved wholly distinct from known pathogenic organisms, would the case for the "new" disease and the transformed parasite be proved? It may, I think, be doubted, in spite even of the incontestable fact that no such nervous disease had until recently come to the attention of physicians, and

notwithstanding chronological relations to the recent pandemic of influenza, no corresponding epidemic disease attended the influenza outbursts of 1889 to 1892. Yet when the older records of epidemics, of which some certainly were and others might have been influenza, are searched as has been done so laboriously and interestingly by Crookshank, there are revealed associated occurrences of nervous or paralytic diseases to which one might hesitate to deny similarity with or relation to the epidemic encephalitis of the present time. As one holding the view that epidemic influenza and epidemic encephalitis are distinct entities, I wish nevertheless to put these facts before you. It cannot, moreover, be entirely without corroborative weight that although epidemic influenza ceased to prevail in this country in 1922–1923, yet epidemic encephalitis still marches on. The two diseases are surely not united specifically: whether they are engendered within a particular constitutional cycle, as Crookshank's argument implies, may be regarded as a hypothetical question which perhaps the experimentalist may ultimately be able to solve.

Let us therefore, for the time being rest the case from the standpoint of human epidemic disease just here. If I have hurried you along rather breathlessly in epitomizing the present state of epidemiology, as ordinarily conceived, it is because I wish to lay before you a possible new mode of approach to some of its problems which, up to now, has been perhaps less invoked than it deserves. I refer to the study in relation to causes and effects of the epidemics occurring among animals to which we have ready access. Now all animals, even the most lowly, and plants of course, suffer from epidemic diseases. There is good reason for believing that in essential respects the happenings are comparable in all. Just the other day I read an account of an epidemic which swept through and destroyed most of that devastating pest, the Mexican bean beetle—but not all—in one of the southern states. This imperfection of extermination is a characteristic of all epidemics, even the severest of man and animals. There is always a remnant of saving grace in the worst outbreaks.

The second or experimental part of this lecture deals with epidemics among mice and rabbits, the study of which has occupied us at the Rockefeller Institute for five years or more. I am led to report these studies in detail because I believe that they help us to comprehend better the events occurring in human epidemics. Doubtless in time epidemics among still other, perhaps still smaller animals, which can be numerously assembled, will come to be investigated. How far down the living scale we must go before we reach the limit of epidemics is not yet determined. Many of you know

that d'Herelle, who has conducted remarkable investigations of the dissolving effects on bacteria of his so-called phage, conceives the active agent as a living virus—a kind of still smaller parasite within the bacteria affected. Although this happens not to be the generally entertained view of the undoubted phenomenon of bacterial autosolution, yet the event serves to recall the satiric lines of Swift, which venture to quote in this connection:

> So, naturalists observe, a flea
> Has smaller fleas that on him prey;
> And these have smaller still to bite 'em;
> And so proceed *ad infinitum*.