THE SPREAD OF BACTERIAL INFECTION; SOME GENERAL CONSIDERATIONS.

By W. W. C. TOPLEY.

(*From the Department of Bacteriology and Preventive Medicine, University of Manchester.*)

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(With 2 Text-figures.)

In a recent series of papers, Flexner (1922), Lynch (1922), Amoss (1922 *a* and *b*) and Webster (1922 *a* and *b*) record the results obtained at the Rockefeller Institute in an experimental investigation of epidemics among laboratory animals. These experiments bear a close similarity to those which have been recorded in previous papers of the present series (Topley, 1919, 1921 *a* and *b*, 1922 *a* and *b*, and Topley, Weir and Wilson, 1921). Their publication affords an opportunity of comparing the results obtained in two independent enquiries, which have been carried out along slightly different lines, and of describing in more detail certain points in the general technique of our own experiments, which have an important bearing on the significance of the results obtained.

One preliminary point may, perhaps, be emphasised. As stated by Amoss (1922 *a* and *b*), at the time when the earlier observations were made at the Rockefeller Institute no report of my own work had yet been published; so that the almost synchronous attack on the same problem by two sets of workers is merely a fortunate coincidence. There is a slight and obviously unintentional inaccuracy in the reference given in Amoss' statement on this point, which might make it appear that the first record of my own investigations was published in 1921, whereas an earlier paper, reporting a considerable series of experiments, was published in 1919. This paper is, however, referred to by Webster in the same series of reports, and the exact date is of little importance. The investigations were, at the start, quite independent. For that reason, perhaps, they afford all the better material for checking or modifying any conclusions arrived at.

However independent in their inception, it is clearly impossible that investigations, so closely connected as regards their subject matter, should long continue in mutual isolation.

It is happily the intention of Flexner and his co-workers to continue and extend their enquiries, and we hope to do the same as regards our own. While the independence of the initial investigations in this unexplored field of research has the obvious advantages which have been referred to, it inevitably results in some little incoherence in any reference to the published records. The same or very similar results will frequently be arrived at by different

workers, travelling by different routes, at very nearly the same time. The exigencies of authorship and of publication will, perhaps, give to the papers when they appear, and in so far as they contain commentaries on the work of others, the effect of a correspondence carried on by a series of letters which are constantly crossing in the post.

Bearing these facts in mind, we may consider in what respect the results so far recorded in the two series of experiments, and the conclusions drawn from them, tend to confirm or refute each other. It may be stated at the outset that the results obtained, by somewhat different methods, are so concordant that the main facts are nowhere in dispute. There is also close agreement on the general nature of the factors, which must be considered in any attempt to form a generalisation based upon the evidence available. Where differences occur, they are mainly concerned with the relative emphasis laid on the various factors considered.

The most fundamental fact so far established is, I think, the following. If a mouse-population be selected, which is in such a state of equilibrium with a bacterial parasite that occasional deaths are occurring, or have recently occurred, referable to infection with this parasite, then the addition of considerable numbers of susceptible individuals of the same species is almost constantly followed by a larger or smaller outbreak of the disease in question. This outbreak presents a wave-form, as judged by a mortality-curve, and almost always leaves a certain proportion of survivors, which react in the same general manner to the addition of fresh susceptibles.

Clearly, the conditions which determine infection or its absence, death or recovery, vary in some orderly manner, and such variation may be initiated by the admixture of susceptible and infective hosts in suitable proportions. Can we offer any probable explanation or analysis of the process which underlies the phenomena observed?

VARIATIONS IN THE CHARACTER OF THE PARASITE.

In his introductory paper Flexner (1922) refers to this matter in the following terms: "Not only do specific differences exist among so called mouse typhoid bacilli, but all the pathogenic varieties appear extremely labile. Strains of the bacilli artificially enhanced as they pass from mice to mice quickly fall to an average of infectivity and are, as it seems, at low pathogenic ebb at the time of the death of the infected animals. But this lability of the bacilli is determined, in part, by the hosts; that is, the mice through which they pass. In this respect mice may be viewed as consisting of different biological classes according as they respond to ingestion of the bacilli with infection and death, with mere carriage of the bacilli, or with non-reactibility."

In the earliest report of the present series (Topley, 1919) the conclusion was reached that "Though reasons have been given for believing that the outstanding feature in the subsidence of an epidemic is a loss of infectivity by the bacterial virus, yet the resistance of the host cannot be a negligible

factor. It will operate by decreasing the concentration of susceptible individuals, and hence the chances of successful transference. It may clearly play an important part in contributing as an environmental factor to the progressive variation of the parasite."

So far, then, there appears to be fairly close agreement in the inferences from the observed facts. It is when we attempt to define more closely the nature of those variations which are especially concerned, and to assess the importance of this factor in relation to others, that the real difficulty arises.

In the paper already referred to (Topley, 1919), an attempt was made to distinguish sharply between the property of virulence, as measured by the rapidity of multiplication of bacteria within the tissues, and the power of producing infection by transference from one animal to another under natural conditions. For the latter property the term "infectivity" was employed, and experiments were quoted, which appeared to show that "the parasite does not cease to be infective because it is no longer virulent...an almost completely non-infective strain—that is, one which produces no illness when taken with the food—may be fully virulent when inoculated into the peritoneal cavity."

Certain passages in one of the contributions by Amoss (1922 *b*) to the recent series of reports from the Rockefeller Institute would, however, seem to show that this point was not sufficiently emphasised. And, indeed, the looseness and inadequacy of our present terminology raise serious obstacles to clearness of expression in discussing the points at issue.

The term "virulence" is very generally used to denote the power of growth, and especially of rapid and widespread growth, within the tissues. There are, however, at least three important objections to this use. Firstly, there is no real uniformity in the meaning attached to this term by different workers. Secondly, its derivation encourages its use in describing any bacterial strain which produces fatal results in natural or in experimental infection. Thirdly, the firmly established custom of differentiating diphtheria bacilli into virulent and avirulent strains, instead of toxigenic and non-toxigenic, makes it very difficult to enforce a strict use of the term.

Similarly, although it was recognised that "As commonly employed, the term 'infectivity' denotes an attribute not of the parasite but of the diseased person, an attribute, moreover, which depends on the patient's environment quite as much as on himself," yet the extreme difficulty of using this term in a logical and consistent manner, when applying it to a bacterial parasite, was not fully realised.

The addition of new terms to our nomenclature is not lightly to be undertaken, but there seems so little hope of attaining clearness of expression under the present conditions, that I would suggest the following changes in our present terminology. The term "virulence" should be discarded, except as a loose description, when it would correspond in all essentials to the term "pathogenicity." The term "infectivity" which I have previously employed

to describe a property of a parasitic organism, should be used only in reference to the infectivity of an animal-host, under conditions which are specified. Three new terms should be introduced to describe (*a*) the power of multiplication on the external and internal surfaces of the body, using a term which would imply rapidity of multiplication and of surface spread, and which could be qualified by such adjectives as "high" and "low," (*b*) a similar power of multiplication and spread within the tissues, and (*c*) the power of breaking through the barrier normally opposed by an intact epithelial surface. As possible candidates for these vacant places in our existing terminology, I would suggest the word "supragliscence"[1] to express multiplication on a body surface, "intragliscence" to express multiplication within the tissues, and "perfringence"[2] to express the production of a breach in a protective membrane. It is needless to add that such terms would have little significance if the conditions in any given case were not specified, but if this were done it seems possible that much would be gained by a strict employment of them. At present it is only intragliscence, or growth within the tissues, that can be measured with any approach to accuracy, but enough is known of the phenomenon of supragliscence, or multiplication on body surfaces, to enable us to attach a meaning to quantitative expressions of the "more" or "less" order.

Repeating the conclusions arrived at in previous reports, so far as variations in the properties of the parasite are concerned, and applying the terms suggested, we may say that supragliscence, perfringence and intragliscence are all essential processes in the natural spread of infection from host to host. Of these, supragliscence and intragliscence can certainly vary independently. Such evidence as is available points to variations in supragliscence as an important factor in determining the observed fluctuations in the epidemic spread of disease, while the part played by variations in perfringence and in intragliscence are still to be determined. As regards each of these factors, the course of events in any individual case will clearly be modified by the relative susceptibility or immunity of the host, and as regards supragliscence the influence exerted by the bacterial flora, already in possession, may need careful study.

VARIATIONS IN DOSAGE, OR MASS OF INFECTION.

While there seems little doubt that variations in the biological characteristics of the bacterial parasite play an important rôle in the events, which together constitute an epidemic wave or series of waves, yet it is reasonable to suppose that the relative mass of infection is one of the determining factors at any given moment. Here, again, the inadequacy of our terminology seems to present a formidable obstacle to clarity of expression, especially when considering the results or conclusions arrived at by others, who may attach a somewhat different meaning to many of those terms in current bacteriological use, which have so far defied any attempt at exact definition.

[1] Glisco, -ere: to increase, to spread.

[2] Perfringo, -ere: to break through.

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Certain comments made by Flexner (1922), with regard to the classes into which mice may be divided, according to the manner in which they react to the ingestion of the bacilli, have been quoted above. He continues "The distinctions of classes are not, however, absolute, but are determined, partly at least, by the quantity or dosage of the bacilli. It is this latter factor which plays so conspicuous a rôle in the phenomenon of recurrent epidemic waves superinduced by the introduction of new mice in the replacement experiments described."

Amoss (1922 *b*) pays considerable attention to this question of dosage, and it is hardly possible fairly to consider the position he takes up without quoting the actual words he uses. In discussing the course of events which ensue when normal mice are exposed to infection from others, among which sporadic deaths have occurred without a true epidemic outbreak, he first considers the effect of an exaltation of "virulence." Having referred to this as a possible explanation, he proceeds: "According to the other supposition the effects described do not depend primarily on augmentation of pathogenicity but rather on increase in mass of the bacillus and result not from virulence so much, or alone, as from dosage. This view involves the conception that favourable conditions of growth and multiplication of the bacilli in the exposed mice are the more decisive events. It would seem as if these circumstances did not exist in the first series employed but did arise subsequently among the new exposures. Once, however, they were secured, the growth and multiplication became such as to overwhelm not only the new series but also the older, previously exposed, survivors, among which the death-rate was finally greater than among the new."

In his discussion and summary, Amoss makes it clear that he does not regard variations in the biological characteristics of the bacteria as of no account, but that he believes the importance of dosage to have been insufficiently emphasised. In any discussion of this matter it is an essential preliminary to decide what "dosage" means. The natural meaning to attach to the term would be, I think, "the number of living bacilli ingested by the host," but it seems very doubtful whether this is the precise meaning intended. Amoss (1922 *a*) himself records experiments yielding irregular results when different strains of a given bacillus were consumed by mice in the form of living cultures mixed with milk, that is under conditions in which the dose must have been relatively enormous. Webster (1922 *a*), in the same series of reports, records the results of *per os* administration of mouse-typhoid bacilli both by feeding mice on bread soaked in broth cultures, and by the more exact method of injecting definite amounts of broth culture into the stomach through a stiff silver catheter attached to a suitable syringe. In passing, it may be remarked that the two methods are, perhaps, not strictly comparable. The conditions to which the bacilli are subjected in the stomach are peculiar, and certainly not favourable to multiplication. Some investigators, indeed, have not hesitated to affirm that the lymphatic route of infection

from the mouth and nasopharynx is more important than the more obvious route along the alimentary tract. This is perhaps an unimportant digression, and it would certainly be extremely difficult to avoid inoculating the nasopharynx in withdrawing the tube, but it cannot, I think, be safely assumed that one is better controlling the effective dose of bacilli by intrastomachal injection than by administration by feeding. The point immediately relevant, however, is the verdict on the evidence presented. Webster says, "It is highly probable that under ordinary conditions of propagation of an epizootic among mice by a representative of the paratyphoid-enteritidis group, infection takes place always *per os*, and yet we know that survivors invariably occur. Our experiments show that the survivors have no necessary relation to dosage of a given constant culture, although on the whole the animals receiving the larger dosage are the ones tending to succumb."

It may probably be taken, then, that Amoss is not referring to the actual number of living bacilli ingested, when he makes use of the term "dosage." It would seem that he has a quite different conception in his mind. This conception seems to be one of a relatively more rapid or less rapid multiplication of the bacilli in the alimentary tract of the host-species, leading to a more or less rapid and extensive dissemination of it among the previously non-infected mice. I do not think it is a quibble to suggest that the term "dosage" gives a very inadequate description of the highly complex process involved. The multiplication of bacilli in the alimentary tract of the host is a function of the bacterial activity and of the environment in which the bacilli find themselves, although none would deny that successful infection in any given case may depend literally on the dose of living bacilli ingested. In so far as this process of rapid multiplication of the bacteria upon the mucous membranes of the host (or, using the term suggested above, this rapid supragliscence) is a function of bacterial activity, its importance was emphasised to the full in the first report on my own experiments (Topley, 1919). Employing the term in common use it was suggested "that a saprophytic spread of the micro-organism concerned, associated with an increasing ability to multiply rapidly under the conditions locally existing, might form the earlier stages of the process" (*i.e.* the epidemic spread of infection). The conclusions drawn by myself from these experiments do not, then, appear to differ very widely from those arrived at by Amoss. The difference in emphasis is, perhaps, merely the effect of observing the same facts from a slightly different angle.

THE RELATIVE SUSCEPTIBILITY OR IMMUNITY OF THE HOST-POPULATION.

This problem may be approached from at least two directions. We may endeavour to determine the conditions which are necessary for the propagation of an epidemic wave, or we may attempt to examine any variations which occur in the relative immunity of the individual hosts during the outbreak of disease. The fundamental fact, that a certain concentration of susceptible hosts is an essential preliminary to the epidemic spread of infection, has been

referred to above. The importance of this factor is emphasised again and again, both in the reports on the American studies, and in previous reports of this series. Certain aspects of this question, and especially the characters which may differentiate the immunity possessed by a herd from the immunity possessed by an individual, are dealt with more fully in a separate report (Topley and Wilson, 1923).

With regard to the immunity possessed by those individuals which have passed through one or more waves of disease, the evidence so far available is entirely concordant. Such surviving members of a host-population have a relative, but by no means an absolute immunity. This relative immunity is usually demonstrated only by the fact that such surviving mice tend to outlive, often by quite short periods, those susceptible mice whose addition to the cage has led to a fresh epidemic wave (Topley, 1921 *a* and *b*, 1922 *b*, Amoss, 1922 *b*). The evidence at present available does not enable us to arrive at a definite conclusion as to whether the relative immunity so demonstrated was natural to these individuals, that is, was present before the initial outbreak of disease and was the factor which led to their survival, or whether it was acquired, either wholly or in part, as the result of a non-fatal infection during some part of the epidemic period. Certain facts suggest that the latter explanation is at least partly true. Webster (1922 *a*) records the appearance of agglutinins for the strain of *B. enteritidis* (Aertrycke) employed, as the result of repeated administration *per os*. Amoss (1922 *b*) records the presence of agglutinins in the blood serum of 60 per cent. of 56 survivors from the first epidemic wave in his replacement series. In some of my own earlier experiments (Topley, 1921 *a*), it was found that bacilli of the enteritidis group could be recovered from the tissues of a high proportion of those mice which had survived a considerable epidemic of enteric infection. There is thus some basis for the belief, that the survivors from a given epidemic wave have resisted infection rather than escaped it, and that the relative immunity possessed by them after the subsidence of such a wave has been either acquired, or increased from a lower level, as the results of non-fatal infection.

THE GENERAL TECHNIQUE OF EXPERIMENTS INVOLVING THE SPREAD OF INFECTION.

There are many other points of interest in this important series of papers from the Rockefeller Institute, but it seems better to reserve consideration of individual experiments for future reports, in which they can be discussed in immediate relation to our own results. There remains, however, one cardinal point, with regard to the general purposes of such experiments and the limitations imposed by technical difficulties, which seems worthy of consideration in some detail, since it is fundamental to the whole nature of the enquiry.

Amoss (1922 *b*) in discussing the relation of his own observation to certain results recorded in earlier papers of the present series, says: "We had in mind not the providing of optimal conditions for infection to take place in mice,

but the imitation, if only roughly, of those occurring naturally in man and in laboratory animals, in connection with which epidemics of disease occur." The method he employed to further this end was to rely, for the spread of infection, upon the chance conveyance by the attendant of infective material from cage to cage, during the routine of cleaning and feeding. His records show that the order of appearance of infection in the cages could not be controlled by the order of cleaning, even with rigid precautions as regards the cleansing of the attendant's hands. The impression is, indeed, left on one's mind that cage-to-cage spread is regarded as inevitable, though it would appear (Webster, 1922 *a*) that some later, unspecified alteration of technique enabled this complication to be avoided.

The object of our own experiments has in no sense been the imitation of the conditions existing in natural epidemics: indeed, this is precisely what we have endeavoured to avoid. The study of the spread of bacterial infection as it occurs in nature appears to us to be rendered particularly difficult because, on the one hand, we are forced to rely on the correlation of events in as large a number of instances as we can collect, and, on the other, the number of possibly relevant factors is so large, and so unpredictable, that the conditions necessary for arriving at any valid generalisation are almost unattainable. The value of the experimental method of attack on this problem seems to us to lie in the possibility of limiting the number of unknown factors, and of devising experiments which shall test the validity of any hypothesis tentatively arrived at.

Obviously, the first condition requisite is effective control of the spread of infection from cage to cage. It is hardly necessary to point out that, in the absence of such control, it would be impossible to carry out many of the experiments described in these reports, without providing separate animal-houses and separate attendants, not only for separate experiments, but for different stages of a single experiment.

This difficulty was encountered by us during the first year of our work, and we were quite unable to prevent cross infection from cage to cage by any elaboration of the precautions taken in the ordinary routine of cleaning and feeding. It soon became clear that, if this complication was to be avoided, the cages must not be "cleaned" in the ordinary sense of that term, but that each experimental cage must be treated on lines approximating to those employed in dealing with a bacterial culture. As soon as this was realised, and our technique modified accordingly, accidental cage-to-cage infection ceased to occur, and for the past three years we have been able synchronously to carry out experiments on different groups of mice, and with different infecting agents, with an almost entire absence of accidental infection.

The routine which is adopted is as follows. The cages employed are made of zinc, and have the general construction shown in Figs. 1 and 2[1]. As shown

[1] These cages have been made for me by Messrs Hawksley and Sons, 83, Wigmore Street, London, W. 1.

in these figures, they can be fixed in series, so as to form together a cage of any desired size. Smaller cages, of the same form but without the side pieces, are used for housing small batches of mice, five or less in number.

For each experiment a double set of cages is provided. Each morning a clean set of cages is taken, the necessary amount of food and sawdust is placed

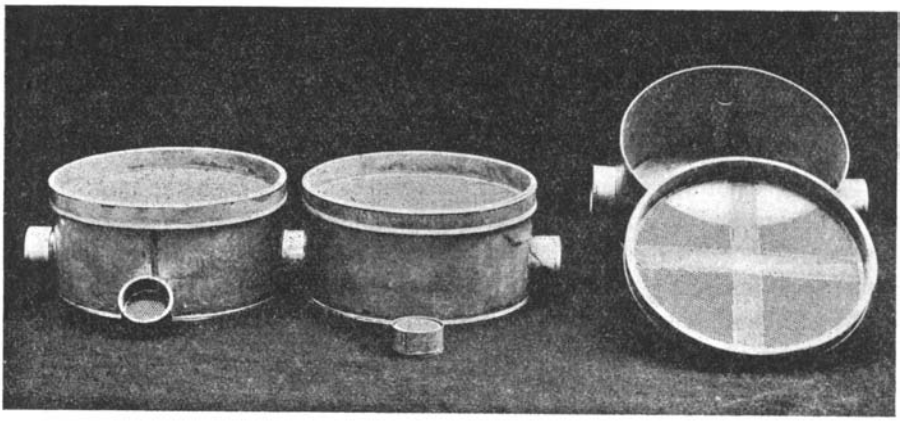

Fig. 1.

in each cage, and the mice are then transferred to these clean cages from those which they are occupying. This transference is carried out by connecting the soiled cage to the clean one, by means of the side pieces, and driving the mice through. If only a few mice are involved these are transferred with forceps.

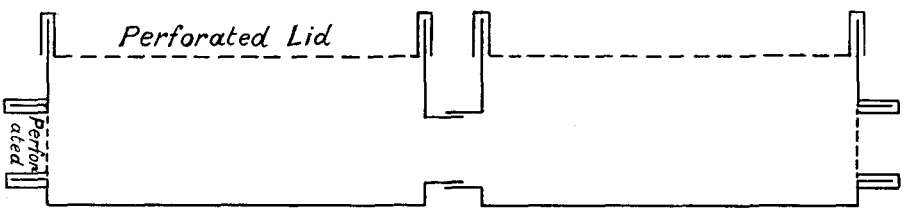

Fig. 2. Diagram of two cages fitted together.

Diameter of cage	= 10 inches.
Height of cage	= 5 ,,
Length of side-piece	= 1 inch.
Diameters of side-piece	= 2 inches and $2\frac{1}{8}$ inches.
Overlap of lids	= 1 inch.
Overlap of covers for side-pieces	= 1 ,,
Distance between lowest part of side-piece and bottom of cage	= $\frac{1}{2}$,,

In either case, all forceps used are immediately placed in a steriliser which is kept constantly boiling during the entire process of transference. Rubber gloves are worn, and are changed as different cages are dealt with, but we believe the essential factor to be the rigorous avoidance of handling the

infected mice, or the interior of the cages, and the immediate sterilisation of all instruments used.

The dirty cages are then removed from the animal-house and immediately placed in a large boiler, where they are sterilised at 100° C. for two hours. After cooling the cages are removed and passed to an attendant who cleans and dries them. They are then placed ready for use on the following morning.

On Sundays no transference is made, the requisite amount of food being added to each cage. The rule rigidly adhered to is that the interior of an infected cage is never touched except with a pair of forceps, which is then immediately sterilised. No other manipulation is carried out until the whole cage and its contents have been adequately sterilised.

The cages employed have certain other features, which are of minor importance. The sinking of the lid, leaving an upstanding rim, lessens the chance of infected litter being thrown through the perforated lid in such a way that it can gain access to other cages. The construction of the covers to the side-pieces has the same object. The cages contain a short tubular fitting, which supports, when desired, a glass tube with a small perforation at the lower end, and fitted with a rubber cork protected by a zinc cover. This is a slight modification of the "abreuvoir" described by Ponselle (1920), and is useful when it is desired to administer fluid unmixed with the solid constituents of the diet.

The normal stock of mice are housed in similar cages, which are very convenient, but these are cleaned by an attendant in the ordinary way. The attendant who cares for the experimental mice also superintends the keeping of the normal stock, and daily selects from them the mice to be added to the experimental cages. We have, however, had no evidence that spread of infection has occurred from the experimental to the normal animals, and we believe that the simple technique outlined will serve to control the direction of such spread. There still remains the case of mice which, when introduced into the experimental cage as apparently normal individuals, are actually infected by some microorganism capable of spreading among the animals already present. This difficulty is theoretically serious; practically it occasionally leads to disaster, but in general it appears to be a factor of minor importance. At all events, it is a factor which cannot be controlled at present.

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