ANTIBACTERIAL ACTIVITY OF AMŒBÆ

THOSE who have followed the development of research on penicillin and other substances which inhibit the multiplication of micro-organisms will be familiar with Sir Alexander Fleming's discovery (*Brit. J. Exp. Path.*, **10**, 226; 1929), that staphylococci cultured on a plate culture failed to develop around an accidental contamination with the mould *Penicillium notatum* and were undergoing lysis, and that culture fluid taken from a culture of *P. notatum* would, even when it was diluted 500–800 times, completely inhibit the growth of the staphylococci. This work, together with other work on penicillin and on various antibacterial substances derived from bacteria and moulds (for example, pyocyanine, gramicidin, actinomycin, aspergillic acid, helvolic acid, etc.), has been summarized in the *British Medical Bulletin* (**2**, No. 1; 1944) and elsewhere. In bacterial cultures in which these antibacterial substances are present, areas in which the bacteria fail to develop appear, which are sometimes called 'clearance areas'. Protozoologists have been aware for many years that certain amœbæ ingest readily certain kinds of bacteria, yeasts and similar organisms, and that they can, under appropriate cultural conditions, produce in bacterial cultures 'clearance areas' somewhat similar to those produced by the antibacterial products of moulds and bacteria. The two phenomena are, however, quite distinct. While the amœbæ clear parts of the cultures by actively ingesting the bacteria, the antibacterial products of moulds and bacteria clear them by inhibition of the bacterial growth, or even by lysis of the bacteria.

The clearance of certain areas of bacterial cultures by the active ingestion of the bacteria by an amœba has been studied by Sir Aldo Castellani (*J. Trop. Med. and Hyg.*, **33**, 160, 188, 221, 237; 1930, and **34**, 83; 1931). Castellani first recorded his study of the active ingestion of the pink yeast, *Cryptococcus pararoseus* (Cast.), by an amœba which was named *Hartmanella castellanii* by M. Douglas (*J. Trop. Med. and Hyg.*, **33**, 258; 1930). Castellani found that pure cultures of this amœba could be obtained by growing it on glucose-agar smeared with the dead yeast. He also found that, when amœbæ were inoculated on to a growth of certain bacteria, after a few days a zone of clearing of the culture appeared which radiated from the point of inoculation of the amœba. Such zones of clearing appeared in cultures of *B. typhosus*, *B. paratyphosus* A and B, *B. dysenteriæ* Shiga and Flexner Y, some strains of *Vibrio paracholeræ* and *B. pestis*, but not on cultures of *B. proteus*, *B. pyocyaneus*, *B. morgani*, *Brucella melitensis*, some strains of *Vibrio choleræ* and all strains of *Staphylococcus* that were tried. The amœbæ sometimes cleared bacteria (for example, *B. coli*), while at other times they did not. Douglas (*loc. cit.*) confirmed these results in the main.

C. E. van Rooyen (*J. Trop. Med. and Hyg.*, **35**, 118 and 259; 1932) studied further this activity of *H. castellanii*. He found that the clearing of the yeast and bacteria by the amœba is due to ingestion of these organisms, and not to a diffusible lysin produced by the amœbæ or to a change in the *p*H of the culture; nor is the action of the amœbæ related to the action of bacteriophage. The rate of destruction of the bacterial culture is proportional to the thickness of the bacterial growth. Van Rooyen confirmed Castellani's discovery that the amœba eats certain bacteria only and will not eat others. He gives a list of about fifty micro-organisms which he subjected to the action of the amœba, most of which are important pathogenic organisms affecting man and domestic animals. The amœbæ were able to destroy, after four days of aerobic incubation at 26-30° C., *B. typhosus*, *B. coli*, *V. choleræ*, *V. paracholeræ*, *B. dysenteriæ* Shiga, *B. pullorum*, *B. suisepticus*, *B. pertussis*, *D. crassus*, *Micrococcus catarrhalis*, *Gonococcus* and *Meningococcus*. To a lesser degree they devoured *B. aertrycke*, *B. suipestifer*, *Streptococcus hæmolyticus*, *B. subtilis* and some strains of *Pneumococcus Type* II. The following were not affected by the amœba: *V. choleræ*, *B. morgani*, *B. fœcalis alkaligenes*, *B. dysenteriæ* Flexner, *Staphylococcus*, *Pneumococcus Type* III, *B. hoffmanni*, *B. xerosis*, *B. pseudotuberculosis ovis* (Preisz), *B. anthracis*, *B. mycoides*, *B. mesentericus*, *B. abortus* (Bang), *B. melitensis*, *B. tuberculosis* (human, bovine, porcine and fish strains and R and S variants of the same), *B. leprœ* (Brinkenhoff) and *B. salmonicida*.

Staphylococcus aureus was used to investigate why the amœba did not eat certain organisms. It was found that substances produced by the staphylococci inhibited the growth and multiplication of the amœbæ. Cultures of *S. aureus* killed and washed in saline were readily eaten by the amœba. The amœba also readily ate killed and washed cultures of the following other organisms which they did not eat when they were alive: *B. proteus* X 19, *B. morgani* No. 1, *B. pyocyaneus*, *B. anthracis*, *B. anthracoides*, *B. leprœ* (Brinkenhoff) and other organisms. The amœba ate mixed cultures provided that these contained either only susceptible bacteria or resistant ones rendered susceptible by killing and washing. The age of the bacterial culture did not make any difference to the ingestion of them by the amœba. The amœba is a strict aerobe and cannot multiply in anaerobic cultures. It will be noted that the bacteria which the amœba will or will not eat do not fall within the Gram-positive and Gram-negative classifications.

Filtrates of saline washings derived from bacteria which the amœbæ will not eat were unable to inhibit the growth and multiplication of the amœba; they did this only when they were combined with the living bacteria and were being produced by them. In certain circumstances the appearances produced by the amœbæ in cultures of bacteria which they will eat resemble closely those produced by bacteriophage action and may be indistinguishable from those of bacteriophage actions except by examination under the low-power microscope. The amœba is extremely resistant to emetine hydrochloride for long periods of time, and prolonged exposure (2 hr. at 75 cm. distance) to X-rays does not kill it.

It seems likely that further work with this species of amœba, and with other species also, would increas the biological interest of these results. It might throw light on the metabolism of amœbæ in general; and, when we remember that the phagocyte is an amœba actively engaged in ingesting and destroying bacteria which are causing disease, the effects of bacterial products on amœbæ in bacterial cultures are perhaps worth further study. It may be argued that studies of a free-living amœba in the presence of pathogenic and other bacteria in artificial cultures will not be applicable to the phagocyte confronted with pathogenic bacteria in the body, but this remains to be proved. At any rate, the work of Castellani

and van Rooyen provides the basis for studies of this kind. While we have, in penicillin and alllied substances, instances of the action of metabolic products of an organism on bacteria, we have, in the work here recorded, an instance of the reverse process—the action of bacterial metabolic products on an amœba physiologically equivalent to the phagocyte, which is not, fortunately for us, affected by penicillin. The work of Castellani and van Rooyen brings us, in fact, nearer to the work on the opsonins and similar substances—work which aimed at rendering the invading bacteria more palatable to the amœbic phagocyte, or at any rate aimed at helping the ingestion of these bacteria by the phagocyte. It is possible that the work done with *H. Castellanii* might be best developed with this idea as its basis.

G. LAPAGE.

AGRICULTURAL SAMPLE SURVEYS

THE need for adequate statistics relating to our agricultural resources and requirements must have become obvious to all during the last few years, for the urgency of war problems has served to direct attention to the inadequacy of peace-time data and also to the methods of rapidly filling the deficiencies. Complete and reliable censuses are often impracticable and always make great demands on both time and skilled labour. Where the need is more for a quick and reasonably accurate estimate of crop acreage, yield, or whatever it may be, sample surveys will generally offer a better method of obtaining the data. These set out to arrive at an estimate of the whole from the collection of a limited sample of representative parts. The dangers of such an approach are as clear as the advantages, and only by conducting the sample surveys along sound statistical lines can biased or distorted estimates be avoided and a measure of the reliability of the estimate be secured.

The need for information of the kind given by sample surveys is, of course, confined neither to Great Britain nor to war-time. In 1937 a statutory body called the Indian Central Jute Committee initiated, as one of its first tasks, a five-year scheme for obtaining improved estimates of the area under jute in Bengal. After some hesitation it was decided to use the sample survey method, the earlier years being devoted to small exploratory surveys, with a complete survey of about 60,000 square miles in 1941. It was laid down that the final estimate of area under jute should have a margin of error not exceeding 5 per cent, that it should be ready early in the jute season and that the cost should not be excessive. P. C. Mahalanobis, who was statistical adviser to the scheme, has now published an account of the methods, both organizational and statistical, by means of which the task was successfully accomplished. The final estimate was within 2·8 per cent of an independent official estimate based on census data; it was ready a week or so before the latest useful date; it cost only about £8,500 as against £110,000 for a complete census. In view of this the Jute Census Committee recommended the adoption of sample surveying to the Indian Government.

Mahalanobis' paper ("On Large-scale Sample Surveys", *Phil. Trans. Roy. Soc.*, B, **231**, 329–451) is divided into three parts. Part 1 describes the way in which the problem arose, outlines the method of approach and discusses production and mapping surveys in addition to those concerning acreage. Part 2 is a mathematical treatment of the statistical theory of various methods of sample surveying. The concepts and principles are dealt with mainly in the abstract, but the results of model sampling experiments are also used. Part 3 concerns the application of this theory to crop area estimation, especially the jute survey of Bengal. The experimental results are summarized and numerical examples worked out.

The fields under jute in Bengal vary much in size, and furthermore any field may be only partly devoted to this crop. It was therefore decided to take as the sampling unit areas, termed grids, of a definite size, like four or twenty acres. The proportion of the land given to jute in each grid was ascertained, and by combining these proportions from all the grids, which were randomly located over the jute-growing area, an estimate of the jute acreage was obtained. Both the precision and the cost of this estimate depend on the area of each grid and the number of grids (that is, density per square mile) surveyed. Now for any given cost, the larger each grid is, the smaller is the density that can be used. The problem is then to adjust grid size, and with it density, so as to maximize the precision of the final estimate. The alternative procedure, which though discussed was not used for the jute survey, is to adjust grid size and number to minimize the cost for a given level of precision.

Two functions, relating cost and precision (variance) to grid size and number, were set up. The constants which they contained were estimated empirically from the data of the early exploratory surveys and by their aid the final survey was planned. The cost function was found to involve consideration of time necessary for enumerating the jute areas within each grid (which depends on grid size but not on density), of time necessary for journeying from grid to grid (which depends on density but not size), of miscellaneous time (independent of both size and density) and of time needed in the statistical laboratory. The precision, or variance, function was found to involve a parameter which took into account the correlation of cropping on adjacent fields. It was also shown that precision varied with proportion of land under jute in the grid, so that the adjustment of grid size and density best for one proportion would not be best for another. For this reason the area to be surveyed finally was divided into zones of more or less homogeneous proportions of jute land, and the best grid sizes and densities found for each zone separately.

Linked pairs of sub-samples, at constant distances apart but randomly orientated, were used to give the standard error of the final estimate. These were always surveyed by different groups of enumerators and at different times, so as to prevent collusion.

The laboratory methods of organizing the survey and randomizing the grids are described in detail, as are the kinds of errors arising from untrained and even dishonest enumerational labour. The means used to adjust the work to the very varied speeds of the enumerators are also mentioned. In the discussion of the planning of sample surveys it is emphasized that surveys of the kind undertaken are progressive. Each one adds to the information relating to the cost and precision functions and their changes with zone and time. So each enables a better survey to be planned for the next occasion. Finally, a detailed account of the work of others on survey sampling is appended.