CLINICAL PATHOLOGY IN GENERAL PRACTICE

LABORATORY CONTROL OF CHEMOTHERAPY

BY

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In the past few years our ability to deal with most bacterial infections has been transformed by the discovery of a series of powerful antibacterial drugs. But knowledge of the correct use of these drugs has not advanced so fast, and there is a danger that, through misuse, many of them may lose their effectiveness or even do harm. The practitioner, knowing that laboratory results are often too late to be of immediate use in urgent cases, tends not to consult the laboratory in those cases in which it can be really useful. The laboratory worker often lacks the experience or imagination to understand the practitioner's difficulties and is apt to criticize him for using drugs blindly without laboratory tests. Yet if these drugs are to be used wisely and safely there is urgent need for clinician and laboratory worker to share their ideas and experience.

Chemotherapy for our present purpose may be defined as "the treatment of infections by specific drugs"—specific both because ideally they are drugs which attack the infecting organism without harming the tissues of the patient and because many of them selectively attack some micro-organisms and not others. In both these ways they differ from antiseptics, though it is not always easy to draw the dividing line. The chemotherapeutic substances in use at the present time fall into two main groups: (1) substances of known constitution prepared by the chemist, such as the sulphonamides, *para*-aminosalicylic acid, isoniazid, etc.; (2) substances which may be of known or unknown chemical composition synthesized by living organisms—the class of antibiotics such as penicillin, streptomycin, chloramphenicol, aureomycin, oxytetracycline ("terramycin"), polymyxin, and erythromycin. The distinction can, of course, be artificial, since chloramphenicol when synthesized by the micro-organism *Streptomyces venezuelae* is classed as an "antibiotic" but it becomes a "drug" or chemotherapeutic substance when made by the chemist.

The laboratory can take part in the control of chemotherapy in the following ways: (1) by identifying the causal organism by direct microscopy and culture; (2) by determining its sensitivity to drugs and reporting the development of drug resistance; (3) by finding out whether adequate concentrations of drugs are being attained at the site of infection or in the circulation; and (4) by preventing or detecting undesirable side-effects.

In this article only bacterial infections are considered, and infections due to protozoa, fungi, and viruses are excluded.

Identification of the Causal Organism

The most useful piece of information the bacteriologist can give is to tell the practitioner *before* he starts treatment what the causal organism is. In a certain number of cases an answer can be given within a matter of minutes or at the most an hour or two. It is often possible by direct microscopy to identify beyond all reasonable doubt the *Treponema pallidum* of syphilis in a primary lesion, the gonococcus in a urethral smear, the tubercle bacillus in

a specimen of sputum, the meningococcus or the tubercle bacillus in a specimen of cerebrospinal fluid. We know that syphilis and gonorrhoea usually respond to penicillin, that cerebrospinal fever usually responds to sulphonamides, and that tuberculosis often responds to streptomycin, *para*-aminosalicylic acid, and isoniazid. The mere identification of the causal organism may therefore give a strong indication of the right drug to use—an indication which clinical judgment alone could not always give.

More often it is not possible on direct microscopy to say what the causal organism is, and we must wait for the results of culture. With most of the common pathogenic organisms cultural results should be available in 24 to 48 hours, though complete identification of a pathogenic species takes much longer. In whooping-cough, cultures may take two to five days. In tuberculosis it is often necessary to wait for several weeks even for the results of primary culture. In such cases the choice of drug has to rest on clinical judgment alone, and the practitioner is tempted to ignore the laboratory altogether. But whenever possible he must make efforts first to obtain a specimen for culture and drug-sensitivity tests, even though he may not be able to wait for the results. These may indicate that a change of treatment is necessary long before failure to respond clinically to a wrong drug is obvious. Repeated cultures during treatment may give useful information about the patient's progress.

It is essential that the practitioner should give details of any chemotherapy the patient is known to have had, since high concentrations of drugs in specimens sent to the laboratory may make cultural work valueless unless special precautions are taken—for example, adding *para*-aminobenzoic acid to antagonize sulphonamides or penicillinase to destroy any penicillin in the specimen.

Sensitivity Tests

From what has already been written it is obvious that for different reasons many infections have to be treated without waiting for the results of drug-sensitivity tests. But properly used these can be of great value in guiding treatment.

As soon as the bacteriologist has obtained a pure culture he can test it for its sensitivity to various drugs which are likely to be useful. In many cases tests of drug sensitivity can be performed on primary cultures inoculated direct from the specimen. This may save valuable time, especially with slow-growing organisms.

There are two methods in common use for testing pure cultures for drug sensitivity—diffusion methods and dilution methods.

Diffusion Methods

If diffusion methods are used, the culture is inoculated on to a suitable solid medium such as a blood-agar plate, and solutions of the drug or drugs to be tested are placed in a reservoir from which they can diffuse into the agar. The reservoir is provided in various ways—by cutting holes in the agar and dropping solutions of the drug into the holes, or by placing on the agar porcelain cylinders, blotting-paper disks impregnated with the drugs, or tablets of the drugs. All these methods are in principle the same: there is a race between the molecules of drug diffusing out from the reservoir and the molecules of nutrients in the medium diffusing into the inoculated organisms, whose fate will depend on the relative speed of these two

FIG. 1

FIG. 2

FIG. 3

FIG. 1.—Penicillin-sensitive staphylococcus showing zones of inhibition of growth obtained with paper disks soaked in penicillin solutions of the following strength: 1,000, 100, 10, and 1 unit per ml. (from the top in a clockwise direction) and a control disk with no penicillin in the centre. The culture has grown confluent over the surface of the nutrient medium except in the areas inhibited by the penicillin. FIG. 2.—Penicillin-resistant staphylococcus. Penicillin concentration, etc., as in Fig. 1. Note that even this organism shows a small zone of inhibition around the disk containing the highest concentration of penicillin (=1,000 units per ml.). FIG. 3.—Antibiotic sensitivity of a coliform organism. The antibiotics are (from the top in a clockwise direction), penicillin (200 units per ml.) and streptomycin, chloramphenicol, aureomycin, and oxytetracycline (200 μ g. per ml.). The organism is resistant to penicillin, but sensitive to the other four antibiotics.

processes. Those micro-organisms which are in a position on the plate close to the drug reservoir may be damaged or killed by the drug before they have a chance to start growing; micro-organisms further away from the drug reservoir may have already grown freely before an inhibitory concentration of the drug reaches them—and then, even if the drug goes on diffusing (as it will if the reservoir is big enough or the concentration in the reservoir high enough) it may be too late, for many of the drugs used are not bactericidal, and even those that are bactericidal often kill bacteria only when they are actively growing. The result is that when the culture plate is examined the next day a zone of inhibition appears around the reservoir of any drug with a strong inhibitory action on the growth of the organism under test.

The size of the zone of inhibition ("no growth") depends, other things being equal, on the concentration of drug used in the reservoir and on the sensitivity of the organism to the drug. The size of zone can thus be used in two ways, either for indicating the relative sensitivities of different organisms when standard concentrations of drugs are used in the disks, or for estimating the concentration of drug, for example in body fluids, if a standard organism of known sensitivity to that drug is used. Methods which involve diffusion from a reservoir cannot be used for accurate quantitative work unless they are carefully controlled, but for rough estimation of the sensitivity of an organism to various drugs they are very useful (see Figs. 1, 2, and 3).

When there is great urgency, time can be saved in two ways: (1) By performing sensitivity tests directly on primary material such as pus or sputum whenever this is likely to contain large numbers of organisms. A control plate free from all drugs must always be inoculated as well, for obvious reasons. (2) By looking for zones of inhibition on culture plates a few hours after inoculation. If these are inoculated early in the morning it is often possible to give a preliminary sensitivity report the same day—a point of great practical value. Such reports are almost invariably confirmed when the culture plates are re-examined the next day.

Dilution Methods

If dilution methods are used a series of dilutions of drug in nutrient medium are inoculated with the organism under test and after a suitable period of incubation the presence or absence of growth in the different dilutions is observed. Fig. 4 shows a typical "titration" of this kind. The culture has grown in the control tube of broth and not in the tube containing 0.015 unit of penicillin per ml., but not in the tubes containing 0.03 unit per ml. or higher con-

centrations of the drug. The organism is therefore said to be "sensitive" to 0.03 unit but not to 0.015 unit of penicillin per ml. of medium.

Dilution methods may be used either with liquid media or, less frequently, with solid media. But to investigate the sensitivity of only one culture to, say, five drugs—penicillin,

FIG. 4.—Dilution method of testing sensitivity of an organism to penicillin.

streptomycin, chloramphenicol, aureomycin, and oxytetracycline—involves a considerable amount of time, and dilution methods in solid media are not commonly used for routine work.

Dilution methods, like diffusion methods, can be used not only for determining the sensitivity of a culture to a number of drugs but also for estimating the concentration of drug in the serum or other fluid.

Reports of Sensitivity Tests

Different laboratories have different methods of reporting sensitivity tests. If diffusion methods only are used it is enough to report cultures as sensitive or resistant to the various drugs tested. Some laboratories go further and report cultures as "highly," "moderately," or "slightly" sensitive or resistant. When dilution techniques are used, the sensitivity of the organism can be reported with some precision. For example, a strain of *Staphylococcus aureus* might be reported as sensitive to 0.03 unit of penicillin per ml. and to the following concentrations of other drugs—streptomycin 1 μ g., aureomycin 0.5 μ g., oxytetracycline 0.5 μ g., chloramphenicol 2 μ g., and erythromycin 0.1 μ g. per ml. These figures can be taken as indicating that the organism is about normal in its sensitivity to the commonly used antibiotics. On the other hand, a strain which needed 100 units of penicillin per ml. to inhibit its growth would be regarded as highly resistant to penicillin. There is some difference of opinion concerning the precise form in which laboratory reports on sensitivity tests should be worded.

The most useful laboratory report is one which gives the results of the tests performed, but also gives enough information to tell the clinician which drugs are likely to be useful in practice.

In most cases the results of *in vitro* sensitivity tests are in harmony with clinical experience. But there are many discrepancies, and not all of them are easy to explain. Tests of sensitivity of bacteria to sulphonamides and to aureomycin, in particular, are apt to be unreliable for various technical reasons.

In tuberculosis most methods of culture are slow, and drug-sensitivity tests involving subculture are even slower, so that it may take weeks to report on the drug sensitivity of a strain of tubercle bacillus from a patient's sputum or cerebrospinal fluid. In patients with positive sputum microscopical examination of the sputum can give evidence of the response to a particular drug or combination of drugs much more rapidly than most of the sensitivity tests at present available.

Drug Resistance

Many a drug has shown great promise at first only to prove disappointing later, because of the rapidity with which resistance emerges in bacteria originally sensitive to it. How does this come about? Several mechanisms have been described. (1) All the individual organisms may be changed by contact with the drug and so become adapted to resist its action. If this were purely a response to environment it might be expected that such resistance would not be permanent, once the drug which stimulated it was stopped. (2) Since it is known that even in the purest of pure bacterial cultures no two bacteria are exactly alike, it is easy to see how, in the presence of a drug, resistant members of the bacterial population could survive its action by a simple process of natural selection. This could occur to some extent whether or not these resistant organisms were true mutants—if they were, the resistance developed would be more likely to be permanent. (3) A culture of predominantly drug-sensitive organisms may be replaced by foreign invaders of a different type from an outside source. Each of these mechanisms is known to operate in some circumstances, and sometimes it is possible that all may be operating together.

The problem of drug resistance is more serious with some drugs and with some organisms than with others. The value of streptomycin is greatly reduced by the ease with which almost all species of sensitive organisms develop resistance—for example, in coliform infections of the urinary tract and in tuberculosis. In tuberculosis isoniazid suffers from the same defect. For this reason neither streptomycin nor isoniazid should be used alone if it can possibly be avoided, and it is now well known that development of resistance can be greatly delayed by the combined use of more than one drug at a time or perhaps in rapid rotation. With penicillin, on the other hand, the development of drug resistance has not proved a serious menace except in staphylococcal infections, and there the incidence of penicillin-resistant organisms has increased greatly, especially in institutions in which there is opportunity for resistant strains to become dominant. With the sulphonamides drug resistance has provided a serious problem in streptococcal and gonococcal infections, yet in meningococcal infections it is seldom important clinically. With the newer antibiotics examples of drug resistance are being more frequently reported as their use becomes more widespread, and here too it seems that staphylococcal infections may present the most serious problem.

Drug Concentrations in Body Fluids

The laboratory is often asked to determine the concentration of drugs in the blood, the cerebrospinal fluid, or other material. Such estimations are of greatest value when new drugs are being tested out for the first time, or when new routes of administration or new preparations of

drugs are being tried. It is worth remembering, however, that, in any particular case, failure to respond may be due to failure of the drug to reach the site of infection rather than to development of resistance, and therefore the measurement of blood levels may be of value—for example, as evidence of whether a patient has in fact been taking a drug at all.

With some drugs chemical methods of estimation are available; with others it is necessary to use microbiological methods similar to those described above for determining the drug sensitivity of organisms, using as standard an organism of known sensitivity to the particular drug concerned. It is obvious that the situation can become very complicated when the patient is receiving more than one drug at a time, and the laboratory work involved may become very time-consuming.

Prevention of Undesirable Side-effects

It is gradually being realized that the powerful chemotherapeutic drugs which are now being used, like all other drugs, can be dangerous either by direct toxic action or by giving rise to a state of hypersensitivity (allergy). Examples of direct toxic action are the deafness and vestibular damage produced by prolonged administration of streptomycin in large doses and perhaps the gastro-intestinal disorders caused by aureomycin. Examples of hypersensitivity are the skin reactions produced by penicillin and streptomycin and perhaps the agranulocytosis or aplastic anaemia produced by chloramphenicol.

The laboratory can sometimes help in preventing these and other dangerous side-effects—for example, by regular blood counts on patients receiving chloramphenicol, and examination of throat swabs, faeces, and other material for the presence of fungi which may replace bacteria and cause fatal complications.

General Conclusions

It is clear that the laboratory control of chemotherapy is no simple matter. There cannot be hard-and-fast rules laying down what kinds of sensitivity tests should be carried out, when they should be performed, and how often they should be repeated during treatment. The need for such tests varies greatly both with the drug being used and with the nature of the infection. Laboratory tests of the sensitivity of organisms to drugs can be misleading, but so can the results of therapeutic trials. Discrepancies between the two can be resolved only if there is opportunity for frequent personal consultation between clinician and laboratory worker.

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Although approved medical schools in the U.S.A. are now accepting their largest freshman classes, totalling nearly 7,500 students, for the fourth consecutive year there has been a decline in the number of applicants for admission, according to an article by Dr. John M. Stalnaker in the April issue of the *Journal of Medical Education*. Of those that apply about 57% are accepted, while of those who apply after rejection the year previously about 40% find places. It is said that the medical schools which limit their applications to State residents in many cases had to "scrape the bottom of the barrel" to secure a freshman class.