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QUALITY CONTROL OF ANTIBIOTICS

by
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Antibiotics have traditionally been considered a class of drugs requiring special control and treatment. This arose largely because they were natural products prepared by fermentation procedures which were technically exacting and difficult to control. In the event of a breakdown in the control of manufacture the potential range of impurities which might be introduced was very great and impossible to define. In addition the natural variability of the micro-organism producing the antibiotic could lead to unsuspected variation in the product.

Although to-day many antibiotics are prepared by more readily controlled procedures of chemical synthesis the form of the specifications of quality applied to antibiotics still largely reflect the type of control which was laid down for penicillin and streptomycin. Pharmacopoeial monographs and other minimum specifications rely largely on a biological assay of potency or activity and a series of tests for non specific toxicity, histamine like activity, pyrogenic activity, and bacterial contamination.

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These rather incomplete "final product specifications" have proved as adequate and acceptable as they have, largely because the products were manufactured by a limited number of manufacturers who were usually the inventors. The companies were usually large ones with a concern for their reputation and in many countries where the antibiotics were produced the national authority exercised a strict control of manufacture through licensing and enforcement of some form of Good Manufacturing Practice. At the present time many of the patents have expired or soon will and new sources of manufacture are arising throughout the world. Often little is known of the conditions of manufacture and it is important to consider what protection is afforded by reliance on final product specifications commonly available.

Probably most weight is put on the biological assay in evaluating a sample of antibiotic or preparation of it. Unfortunately most biological assays of antibiotics have very little specificity, the response being measured is inhibition of growth of a sensitive micro-organism, a single strain of test-organism may be used to assay many different antibiotics. Under ideal conditions bioassay can give a precise estimate of the content of a particular antibiotic in a given sample or preparation but a number of criteria must be satisfied before the assay gives a valid measurement of activity.[1,2] The criterion most relevant to analytical control is that the test substance being assayed and the standard preparation against which it is being compared should have similar compositions i.e. the test material should behave as though it was a dilution of the standard preparation. If standard and test are comparable they will give parallel dose-response lines, if they are not comparable the dose-response lines may be non-parallel. An assay in which the dose-response lines are non-parallel cannot give a relative potency, which is a measure of the content of active material in the test sample since the sample does not have a unique potency value. The lack of identity of composition of test and standard is the main source of difficulty in evaluation of potency of antibiotics.

If the assay is valid it is still subject to the effects of biological variation and the precision with which the potency is determined may vary greatly from assay to assay. It will depend among other things on the degree of uncontrolled variation and the extent of replication of the responses measured. Confidence Limits can be calculated from the date of the assay which provide a probability to the range within which the true potency may be. To avoid fruitless arguments between a manufacturer and a checking laboratory (national control laboratory) the manufacturer should not release a batch of "antibiotic" unless the lower Confidence Limit of assay is greater than the minimum permitted potency, the Control Laboratory should not reject a batch of "antibiotic" unless the upper Confidence Limit of its assay is less than the minimum permitted potency. It will never be possible to control biological potency of an antibiotic with the precision expected of a chemical or physical assay but if assays are performed with Confidence Limits of at least $\pm$ 5.0% then it should be possible to ensure that the patient receives at least 80% of the intended dose.

This control of active constituent will, however, only hold true if the preparation contains the antibiotic it is meant to contain, a fact which the assay does not demonstrate. When the history of the preparation is known through a proper application of in process control as part of "Good Manufacturing Practice" then the assay may be reliable. For antibiotics, particularly in final dosage form, when the history is not known with certainty a positive identification is most important in view of the non specificity of the bioassay. Simple reliable tests are rarely to be found in available monographs and it may be necessary to use a combination of different techniques e.g. spectrophotometric; infra red analysis, electrophoresis, chromatography to make a positive identification.

As mentioned previously most official "final product specifications" for dosage forms found in pharmacopoeias or regulations such as those of the FDA are based on an assumption that proper material has been used and perhaps more attention should be given to the inclusion of specific identification, in the final product, in official monographs.

The need for a more stringent control of the quality of the final dosage form has been recognized in relation to tetracycline. This is one of the only known antibiotics where degradation of pure material can lead to the development of a toxic impurity e.g. epianhydrotetracycline. This degradation could occur in the final dosage form if an unsatisfactory formulation was combined with improper storage and there is a real need for tests on the final dosage form to limit this impurity. This danger has been recognized in the UK by the inclusion of tests, to limit the content of epianhydrotetracycline in Tetracycline Hydrochloride in the 1969 Addendum to the BP 1968 and by similar proposals made by the FDA[3]. The tests of the BP rely on a Thin Layer Chromatographic separation and semi-quantitative measurement of epitetracycline, anhydrotetracycline, and of epianhydrotetracycline to not more than 0.5%. This test is supported by a Light Absorbing Impurities test which will limit total anhydro content e.g. epianhydrotetracycline and anhydrotetracycline to not more than 1%. Tablets, capsules and injection of tetracycline of the BP are also now subject to a Light Absorbing Impurities test limiting total anhydro content to 2% for tablets and capsules and 1% for injection. The importance of improving identification procedures was recognised by the introduction of a TLC test of identity into the BP 68 and Add 69 for tetracycline as a group.

The TLC test described in the BP Addendum 1969 for limiting the degradation impurities of Tetracycline Hydrochloride should allow a competent analyst to examine tablets and capsules of this antibiotic and decide whether or not the tetracycline hydrochloride contained in the dosage form is of pharmacopoeial quality but a certain amount of preliminary examination would be necessary in each case to establish a valid procedure. This situation underlines the need for control of quality through implementation of "Good Manufacturing Practice" by the national authority licensing the particular manufacturer. This national authority could then require each manufacturer to establish the valid procedure for his particular formulation of tetracycline.

As the minimum accepted purities for different antibiotics are raised and approach closer to theoretical purity it becomes more necessary to rely on chemical and physical evaluation instead of biological assay. It is impracticable to perform bioassay with a precision better than ± 2% and therefore difficult to use bioassay to evaluate an antibiotic with a minimum potency equivalent to 95% purity or better. For this reason many official specifications no longer rely on biological assay for evaluation of the various penicillins, although bioassay may be used for convenience in the assay of formulated products where less precision is needed. It is likely that a similar change may become acceptable for the tetracycline group in the next few years.

Antibiotics which probably produce the most problems in analytical control are those which are mixtures of biologically active homologues e.g. neomycin, bacitracin, nystatin, polymyxin. Published specifications for these are most primitive and the problems which arise through disagreement over potencies of particular batches of material cannot be avoided until chemical and physical methods of analysis are used to measure the proportions of the different components and until manufacture is controlled to produce material with a restricted composition. Methods are readily available which allow the proportions of neomycin B, neomycin C and neamine in a sample to be determined but commercial neomycin complex circulates in the market with a content of neomycin C varying from 2% to 30%. There does seem to be a need, here, to specify two grades of neomycin. (1) designated "neomycin B" containing perhaps not more than 3% neomycin C and (2) "neomycin complex" containing perhaps not less than 10 or 15% and not more than 25 or 30% of neomycin C. Assays would be performed in the first case in terms of a standard preparation of pure "neomycin B" and in the second case in terms of a standard preparation containing 20% of neomycin C and 80% neomycin B. The pure grade of "neomycin B", which could be assayed precisely, could be used for those therapeutic purposes e.g. injection into body cavities where precise dosage is necessary; the grade designated "neomycin complex", for which assays would be less precise, would be used for those purposes e.g. topical and oral administration,

where precise dosage is less important. Although assays of the complex would be less precise they would probably be much less variable than at present. An international collaborative study of a proposed international reference preparation of neomycin B is at present in progress[4].

Readily usable methods of analysis of the homologous biologically active constituents of bacitracin, polymyxin and nystatin are not at present available and it is unlikely the problems of variability in assay will be resolved until they are.

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